

THE DEVELOPMENT OF THE STERNUM IN *SUS SCROFA*

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NINETEEN FIGURES (SIX PLATES)

1. INTRODUCTION

There is a fairly large and scattered literature upon the various phases and problems of the mammalian sternum. Most of this, however, consists of more or less incidental references or descriptions of but one or more stages. So far as I am aware, no one has ever given a complete account of the sternum in a single animal from its earliest appearance in the mesenchyma to its final condition in the fully matured adult. In spite of the vast amount of work that has been done upon that ubiquitous laboratory animal, the pig, but very little notice has been taken of its sternal apparatus. The pig, therefore, furnishes all the material for this investigation.

Flower ('85) gives one small and very poor figure of the sternum of the adult pig, together with the following description: "The presternum (manubrium) is compressed and keeled; the articular facets for the first pair of ribs are close together on its upper surface; but the mesosternum (gladius) is broad and flat, the first segment being transitional, compressed in front and broad posteriorly. The xiphisternum is narrow and pointed. The sternum of the pig very often retains indications of the primordial median fissure through life."

Parker ('68) figures the sternum in an embryo pig 4 inches long and in the adult. He gives two figures of the first stage and one of the second. In the embryo figured, the sternum and costal cartilages are entirely cartilaginous; while in his figure of the adult, ossification is completed. We have, therefore, no

intimation from Parker as to the earliest precartilaginous stages, and neither the when, where, nor how of ossification.

Kravetz ('05) and Whitehead and Waddell ('11) used early stages of the pig embryo in support of their theories of sternal origin. Minot ('92) makes a casual reference to the sternum of the 50-mm. pig embryo. Rathke ('38) was the first investigator to accurately describe the sternal bands of a mammal.

2. MATERIAL AND METHODS

The material for this study consisted of a series of transverse sections of pig embryos ranging in size from 16 mm. to 30 mm. Embryos from 50 mm. to the full-term fetus were dissected and the sternum and costal cartilages removed. A series of sterna ranging in age from birth to an old adult were kindly supplied by the St. Louis branch of the Swift Packing Company. As the usual process of butchering bisects the sternum longitudinally, these sterna from young and adult hogs had to be taken out by a special process, for which trouble and expense my deep appreciation is expressed to Mr. A. J. Green, superintendent of the Swift Packing Company. The drawings are the work of Miss Bertha L. Uhlemeyer.

3. DISCUSSION OF STERNAL ORIGIN

According to Whitehead and Waddell, the youngest stages of the pig in which any sternal anlage could with certainty be detected are those of 15 to 16 mm. in length. The sternal bands stopped posteriorly at the level of the third and fourth ribs, respectively. In each case the first two pairs of ribs failed to reach the bands. No anterior or presternal anlage was present.

In the 18-mm. stage the sternal bands extend from a point 150 μ anterior to the level of the first ribs back to the seventh rib. Again, the first two ribs fail to reach the sternum; the anterior anlage is still absent; and the position of the pericardial cavity and heart far forward into the neck results in the widely separated sternal bands. Whitehead and Waddell also describe embryos of 20 mm., 22 mm., and 24 mm. in length, but as my

first stage (24 mm., fig. 1) is not essentially different, its account is taken up at this point.

As will be seen from figure 1, a presternum is present for the first time and unites the anterior ends of the sternal bands. The rapidly sinking heart has permitted the sternal bands to approach each other anteriorly, while posteriorly they are still widely separated. It is clearly apparent in the sections that the ventral end of the first rib, which is still mesenchymatous, does not reach the sternum; the relation of the second is uncertain, while the remaining five are firmly fused with it. Both Kravetz and Whitehead and Waddell describe a 24-mm. stage. Kravetz found that the first rib did not reach the sternum, while Whitehead and Waddell say that it does. In the specimen studied by Kravetz, the remaining six pairs of ribs were too feebly connected to the sternum to have any morphological significance, but Whitehead and Waddell say that the connection of the posterior six pairs of ribs is marked. My 24-mm. stage agrees with that of Kravetz in that the first rib does not reach the sternum, and with Whitehead and Waddell in that the connection of the remainder (the second excepted) is marked. No special significance attaches to these variations, since differences of the parts in development as well as in measurements and amount of shrinkage give room for considerable variation.

It is the opinion of Whitehead and Waddell that a stage must exist in the pig in which no ribs reach the sternum. It must be admitted that no one has ever been able to find such a stage in pig material; however, in other mammals, man, rat, mouse, and cat embryos, such stages have been found. This is obviously an important item, for if once clearly demonstrated that the sternal bands exist as separate structures prior to their union with the costal cartilages, Ruge's ('80) theory of sternal origin would no longer be possible. Ruge's view that the sternal bars are derivatives of the ventral ends of the costal cartilages is generally accepted to-day and has found a place in nearly all comparative anatomy and human anatomy texts. This theory was first attacked by Paterson ('00, '02, '04), who worked upon the rat, and found in that animal a complete girdle comparable

to the pectoral girdle of the dogfish. The ventral midportion of this he identified as a presternum and claimed that the sternal bars were backward prolongations of this anterior median sternal anlage. In this latter observation Paterson was undoubtedly in error, for I have recently studied this region in the mouse, cat, pig, and man, and agree with Whitehead and Waddell that the sternal bands antedate in appearance the single anterior presternal anlage. Paterson, however, rendered a valuable contribution to the discussion of sternal origin by pointing out, first, that the presternum and coracoids were in intimate union in mammals in the mesenchyme stage, just as they are in the young and adult stages of many lower vertebrates; and, second, in pointing out that the sternal bars are separate entities before attached to the ribs, which relation thus becomes a fusion of secondary importance.

That in the ontogeny of the mammals the sternal bands should antedate the presternum presents a contrary procedure to what is found in the phylogeny or evolution of the sternum in the vertebrates. In the lowest amphibians the presternum is developed first, while the sternal bars are added later in the highest amphibians and the reptiles as backward prolongations of the presternum. Naturally, the development of the sternum in a mammal would be expected to follow the order of its evolution, presternum first, sternal bands later. That it does not do so is probably due to the position of the heart, which is far forward in the neck in a young stage and has a great ventralward extension. This causes the wide separation of the sternal bands and inhibits the appearance of the median sternal anlage until the subsidence of the heart into the thorax. This illustrates a corollary to the law of recapitulation, namely, that a retardation of the development of a structure may occur so that it does not appear in its proper sequence in ontogeny.

Whitehead and Waddell reject both Ruge's theory of costal origin and Paterson's theory of coracoidal origin for the mammalian sternum, suggesting that from the history in the forms they studied (pig, cat, and man) the sternum arises independently of any other structures. Thus, at the present time there are ex-

tant in the literature three theories of sternal origin. In another paper, soon to be published, I have discussed critically these three theories in the light of all the evidence obtainable from the fields of both phylogeny and ontogeny.

It will suffice here to say, that the evidence compiled by Paterson, Whitehead and Waddell, Kravetz, and myself against Ruge's theory is overwhelming. The sternal bands have been shown to be entirely independent of ribs, and appear in development prior to the ventralward growth of the ribs. Ruge does not appraise the fact that the sternebrae are relatively late in appearance, are invariably intercostal and their centers of ossification always occur in that portion of the sternum between the ends of the ribs. These latter facts are illustrated by figures 8 to 13, inclusive. According to the Ruge hypothesis, the sternal bands at their first appearance should consist of a series of short segments opposite the ends of the costal cartilages. By ossification of these a series of sternebrae would be differentiated, whose union would form the sternum. That these are not the facts in the case is attested by all workers on the earliest stages of the sternum.

Concerning the 'in situ' theory of Whitehead and Waddell, it may be remarked that so ancient a structure as the sternum, dating back to the dogfish, *Hexanchus*, and found in that form and in all intermediate groups of vertebrates in the most intimate relation to the coracoids, either in the embryo or throughout life, can hardly be accounted for in the mammals in such a simple fashion.

This leaves the coracoidal theory of Paterson, which is based upon the anterior girdles in the lower vertebrates as being comparable to the complete mesenchymatous girdle found in the rat, mouse, cat, man, and presumably in all mammals with well-developed clavicles. This has much in its favor. In forms like the pig where clavicles are entirely aborted, there never occurs this sternalward extension of the mesenchymatous coracoids, which fact led Whitehead and Waddell to reject Paterson's view.

4. DESCRIPTION OF PIG STERNA

Figures 1 and 2 are graphic reconstructions of the 24-mm. and 30-mm. pig sternum, respectively. Every other section was drawn with the aid of the camera lucida, and the sternal bars and ends of the ribs were plotted upon millimeter-ruled paper. This gives an exact reproduction to scale, and is a quick and satisfactory method for structures which are partly mesenchymatous or just emerging from that condition. The irregularities in the sternal bands are due entirely to the method of reconstruction. The bands themselves are simple, straight, ribbon-like structures, bearing no evidence of segmentation such as would be expected were they costal products. Furthermore, in these early stages there is always a suture or definite line of demarkation between the rib and sternal band, whereas in the later embryos the one structure passes over insensibly into the other until a comparatively late stage, after which the ribs again separate from the sternum by joints. Figures 1 to 5 show the sutures in the youngest stages; figures 6 to 13 indicate the second stage in which no demarkation between rib and sternum is apparent, while the remaining figures show the separation of the ribs and sternum by joints in young and adult animals. On the supposition of Ruge, it should be the youngest stages in which sternal anlage and costal cartilages are continuous.

The fact that in the 24-mm. pig (fig. 1) the first rib does not reach the sternal band has already been mentioned. In the 30-mm. stage (fig. 2) all seven ribs are attached on each side to the sternal bands, which latter structures are more closely approximated in the middle line than in the preceding stage. A wax model also was made from this same pig with like results, but the illustration is from the graphic reconstruction. Figures 3 to 5 are cross-sections from various levels of this stage, for details of which see the explanation of figures.

By gross dissection the sternum of a 50-mm. pig was exposed and found to be in exactly the same condition as the 115-mm. stage (fig. 6), except that the parts were correspondingly smaller and more delicate. Eight pairs of ribs joined the sternum as in

most other fetal pig sterna. Only seven reach the sternum in the adult, indicating probably that in the pig also the posterior end of the thorax is undergoing degeneration. Some interesting variations were found in the number of ribs which fuse with the sternum in fetal life. Figure 12 shows that in this specimen nine ribs reach the sternum, while the tenth pair falls just short of making the connection. In this stage the bands have fully united and the xiphisternum is formed. Kravetz worked out several stages of pig sterna between 24 mm. and 50 mm., so I can pass on to my next stage in the 115-mm. pig.

Figure 6 shows the sternum in this stage (115 mm.) to be a broad, shield-like plate of thin cartilage. The presternum and xiphisternum are well differentiated, the median fissure is indicated by much thinner cartilage than the two sides of the sternum. Eight pairs of ribs are in union with the sternum and are not separated by sutures or joints.

In the 131-mm. pig (fig. 7) the general topographical features are as in the one preceding. However, a large fontanelle is present in the lower portion of the mesosternum (gladius) indicating an incomplete fusion of the sternal bars in this region. This is a character occasionally present in man and other mammals and is a constant feature of some of the reptilian forms. This is the earliest stage in which any ossific centers are present. One single and one paired center appears. It is rather strange that all the centers are not paired, since this shield-like plate is the product of two longitudinal sternal bands. The sternum is relatively late in ossification. In a pig of 33 mm. the ribs have begun the ossification process, while in the 131-mm. stage is found the first indication of a bony deposit in the sternum.

Figures 8 to 13 are fairly close stages from the 133-mm. pig to that of three weeks after birth. They show well the range of variation in number, position, and growth of the ossific centers. Many of these show their originally paired condition. If the presternum is the phylogenetically older structure, and it undoubtedly is, it might be expected to ossify first or at least early. On the contrary, it is the last segment to do so, and there is never a complete replacement of the cartilage by bone. In my

oldest sternum (fig. 19) the anterior end of the manubrium is capped with cartilage and this is proportionately larger in younger stages as seen in figures 14 to 16, inclusive. The xiphisternum always bears a large cartilaginous lobe or tongue of varying dimension and shape.

Figures 14 to 19 are different views of growing and adult sterna. I have still other younger and intermediate stages, but as these exhibit nothing additional they are not figured. The number of ribs has been reduced to seven, the ossific centers of the feti have spread until the sternum is now composed of a number of sternebrae. The number is seven, counting the cartilaginous part of the xiphisternum, and is constant for the pig. The number of sternebrae in this and in other forms corresponds generally to the number of ribs articulating with the sternum. I have shown elsewhere how the sternebrae arise, not as costal contributions, but very late in phylogeny (being found only in mammals) and also late in ontogeny, as the response to a functional demand for as much elasticity on the ventral side of the animal as is allowed by the vertebral column on the dorsal side. Since the ribs are deeply set into the sternum, a series of weak points are produced on either side corresponding in number and position to the ends of the ribs. At these junctions of ribs and sternum the sutures between the sternebrae are produced.

Figures 17 and 19 are dorsal and ventral views of the sternum from an old boar. In two of the sternebrae the primordial median fissure is clearly marked by a suture. Upon sawing the sternum crosswise at this level it was determined that the suture passed through the entire bone and was not merely superficial.

No clavicle attaches to the pig sternum and no trace of an episternal or suprasternal cartilages was observed. The entire absence of suprasternals is probably due to the loss of the clavicles. These two structures seem intimately related, though the homologies of the suprasternals are still in doubt. These small cartilages are constant in the rat and mouse where the clavicles have a full development, and are also occasionally present in man. Huntington ('18) derives them from the interclavicular ligaments.

5. CONCLUSIONS

This study seems to warrant the following conclusions:

1. That the pig sternum cannot be a derivative of the costal cartilages, since it exists as a distinct entity prior to the fusion of ribs and sternum.

2. That it may arise 'in situ,' as Whitehead and Waddell suggest, but more likely its genesis is wrapped up in some way with the coracoidal hypothesis of Paterson, and this study must be approached through the lower forms of vertebrates.

3. That the sternal anlage of the pig first appears as two longitudinal bands on either side of the body; these bands gradually approach the median ventral line; are united anteriorly by the presternum, which does not appear until after the sternal bands, and from these three anlagen, two lateral and one median, the adult sternum is formed.

4. No clavicles, episternum, or suprasternal cartilages have any existence in the pig.

5. The sternal bands are unsegmented in their earliest stages; the sternebrae and ossific centers appear late in development and are always intercostal in position.

6. Ossific centers are often double in each sternebra; that for the presternum being the last to appear.

7. There are normally eight pairs of ribs attached to the sternum in the embryo, which number is reduced to seven in the postnatal life.

8. The primordial median fissure occasionally persists through life, and may or may not be accompanied by an incomplete fusion or fontanelle.

9. The extreme anterior end of the manubrium and the posterior part of the xiphisternum always remain cartilaginous, even in very old specimens.

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PLATES

ABBREVIATIONS

Oc, ossific center

PSt, presternum

R1-8, first to eighth ribs

St, sternum

StB, sternal band

XSt, xiphisternum

PLATE 1

EXPLANATION OF FIGURES

- 1 Graphic reconstruction of sternum of pig 24 mm. Sternal bands are united anteriorly by presternum. The first pair of ribs does not reach the sternum. The second pair merely touches the sternum, while the remaining five pairs are firmly fused with it.
- 2 Graphic reconstruction of 30-mm. pig sternum. All ribs reach the sternal bands, which are approaching each other.
- 3 Cross-section of 30-mm. pig, showing how the first pair of ribs is related to the presternum. Series no. 48, slide 80, section 3. $\times 28$.
- 4 Cross-section of 30-mm. pig, showing union of third rib with sternal band. $\times 50$.
- 5 Section of ventral body wall of same animal through posterior part of sternal band.

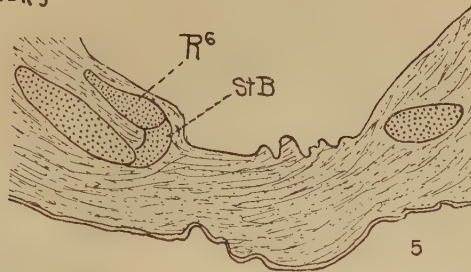
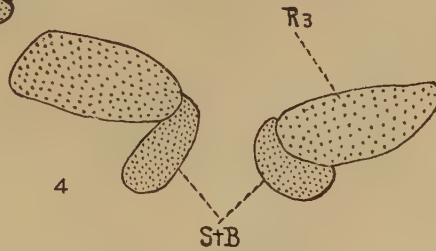
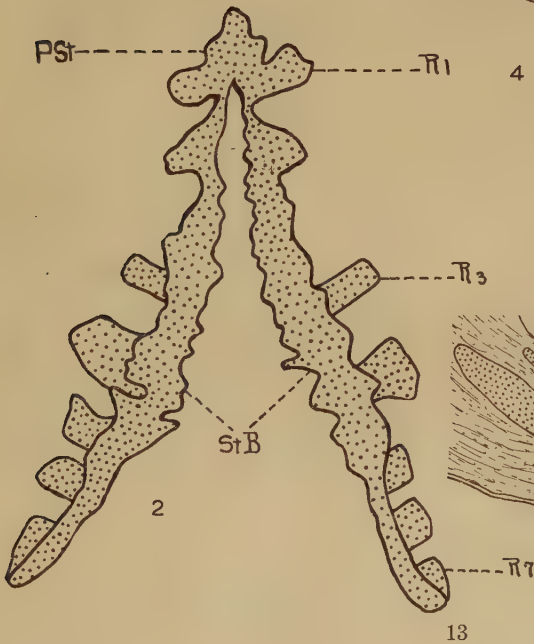
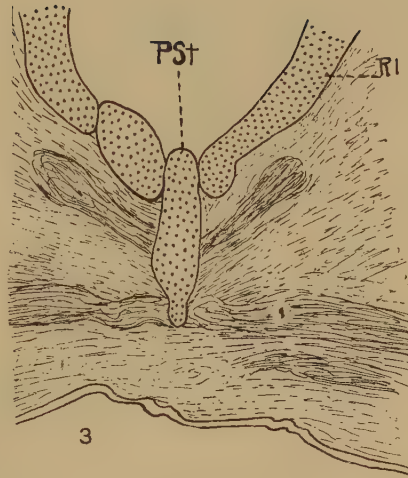
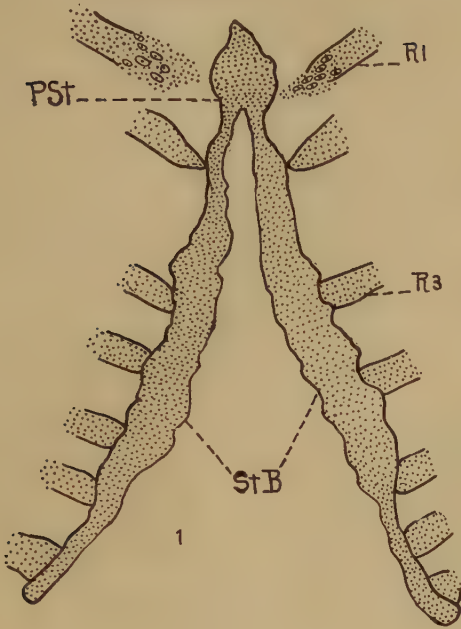


PLATE 2

EXPLANATION OF FIGURES

6 Sternum of 115-mm. pig. Sternal bands fused completely. First anlage of xiphisternum. Eight pairs of ribs attached to sternum.

7 Sternum of 131-mm. pig. First appearance of centers of ossification. One center is paired, the other is single. Lower part of sternum bears a fontanelle, which is occasionally present as a result of incomplete fusion of sternal bands.

8 and 9 From pigs 133 mm. and 150 mm., respectively. Show increase in number and size of ossific centers. Figure 9 shows an accessory center of ossification near the large center for the xiphisternum.

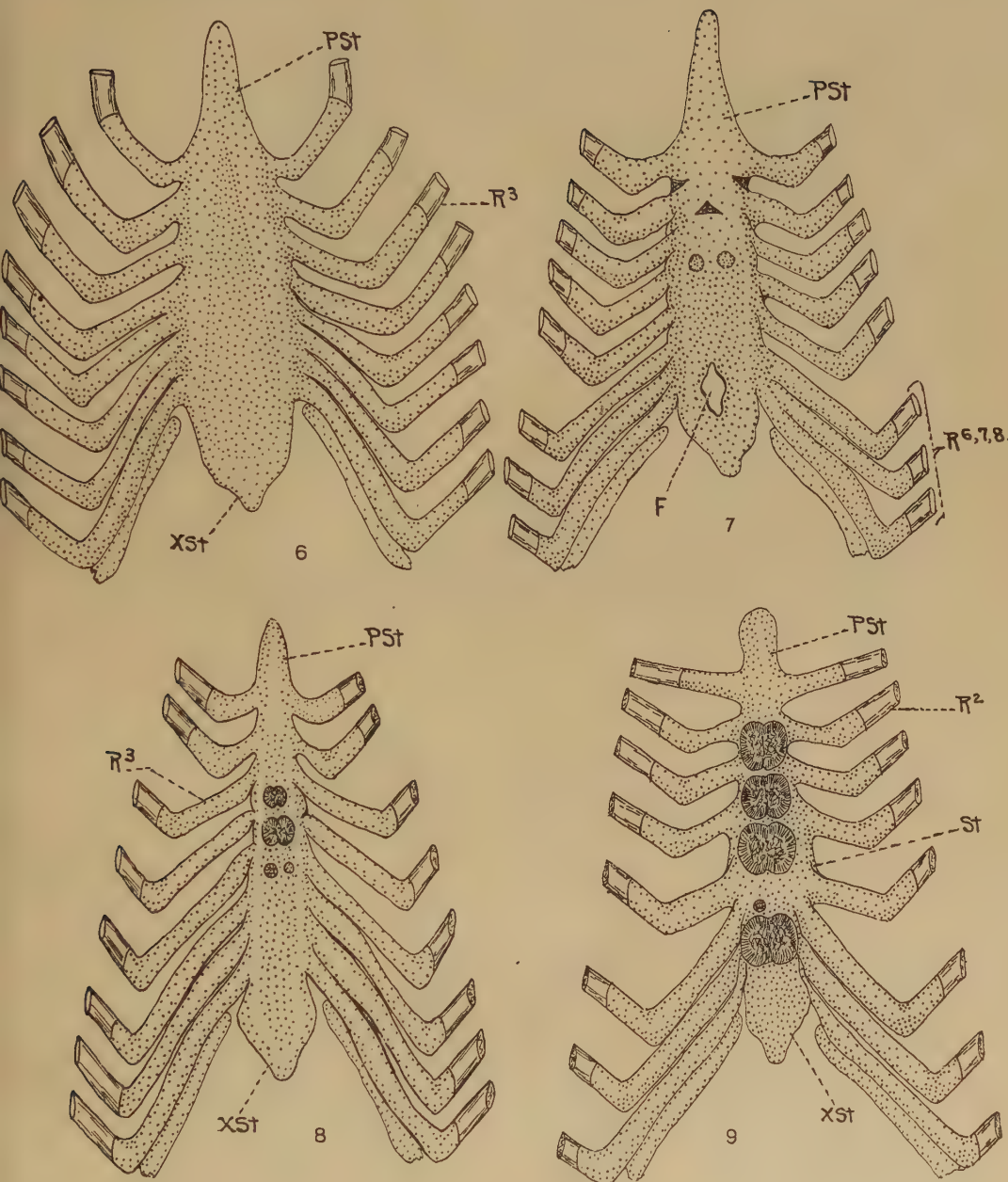


PLATE 3

EXPLANATION OF FIGURES

10 to 13 Pig sterna from animals 177 mm., 200 mm., the ripe fetus, and 3 weeks old, respectively. Figure 12 has nine ribs attached to sternum and figure 13 shows how posterior ribs are cut off from sternum, reducing the number to seven in the adult.

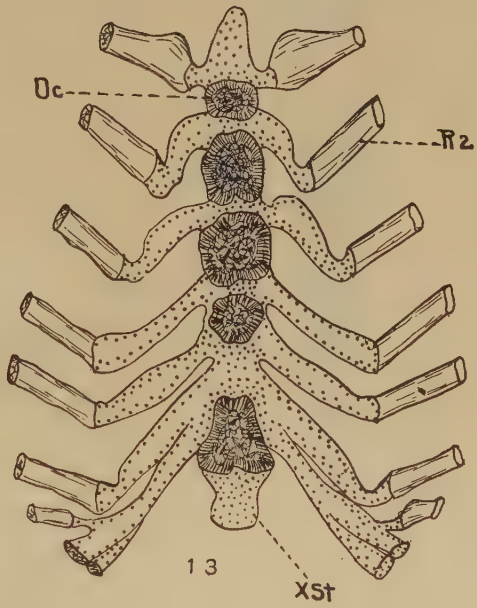
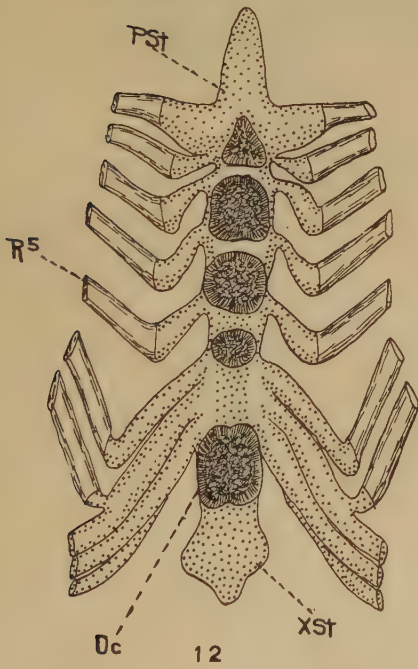
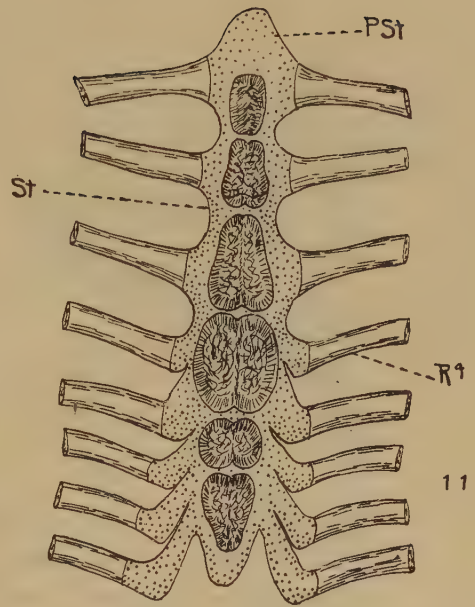
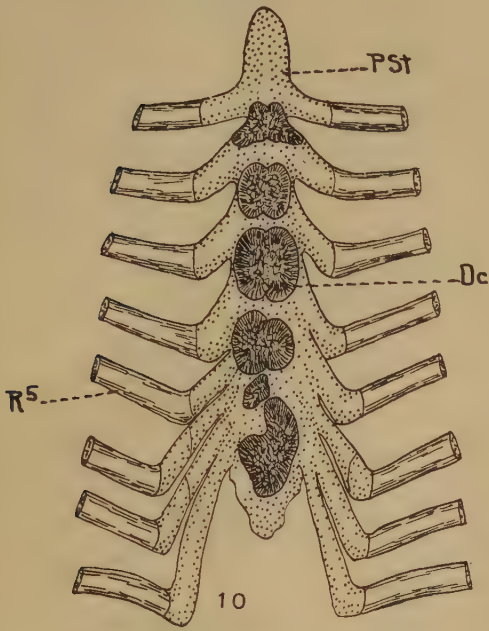


PLATE 4

EXPLANATION OF FIGURES

14 to 15 Ventral and lateral views of a sternum from a pig nearly three-quarters grown. Ribs have separated from sternum by joints. Sternebrae well ossified, separated from each other by cartilaginous pads.

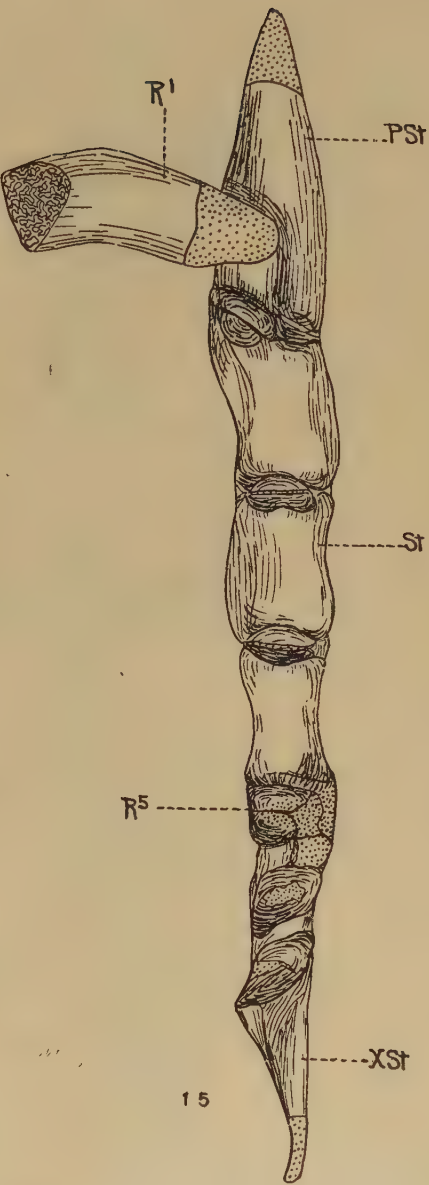
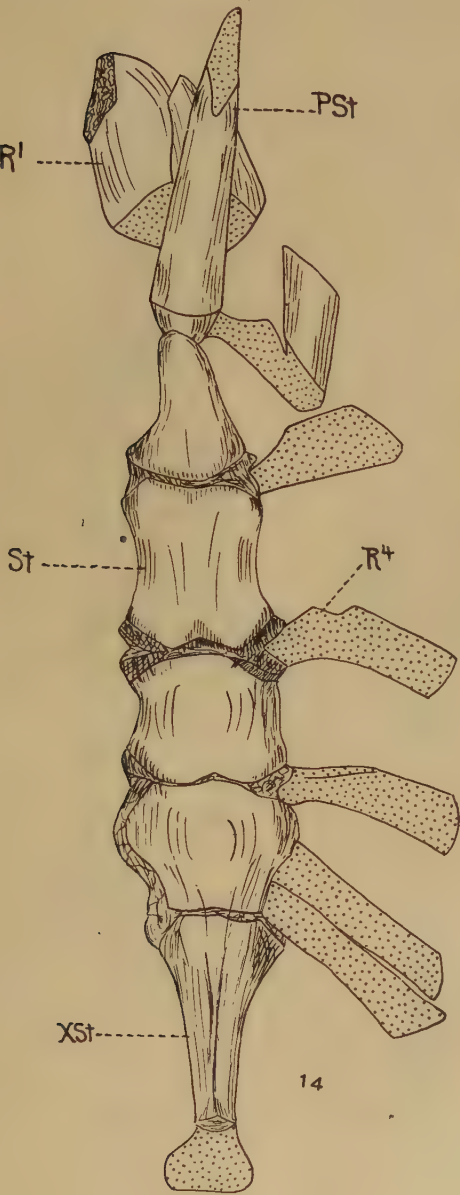


PLATE 5

EXPLANATION OF FIGURES

- 16 Dorsal view of sternum of young adult pig.
- 17 Dorsal view of sternum of old boar, weight 450 pounds, age unknown.

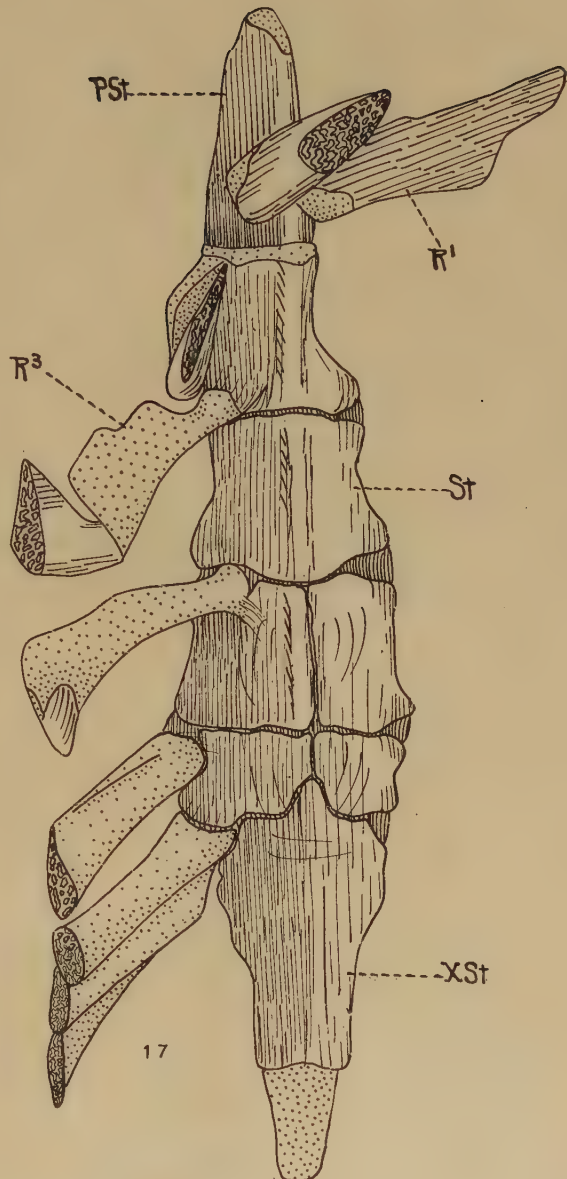
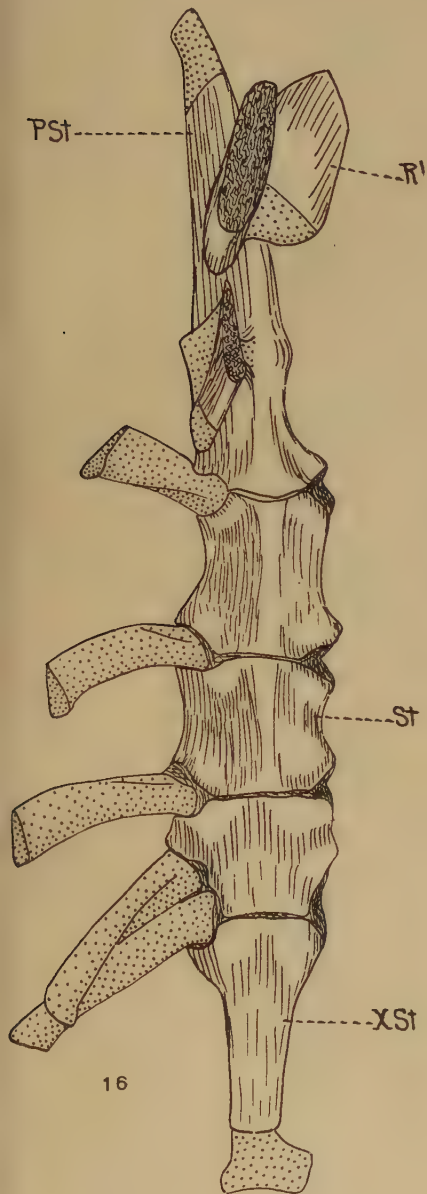
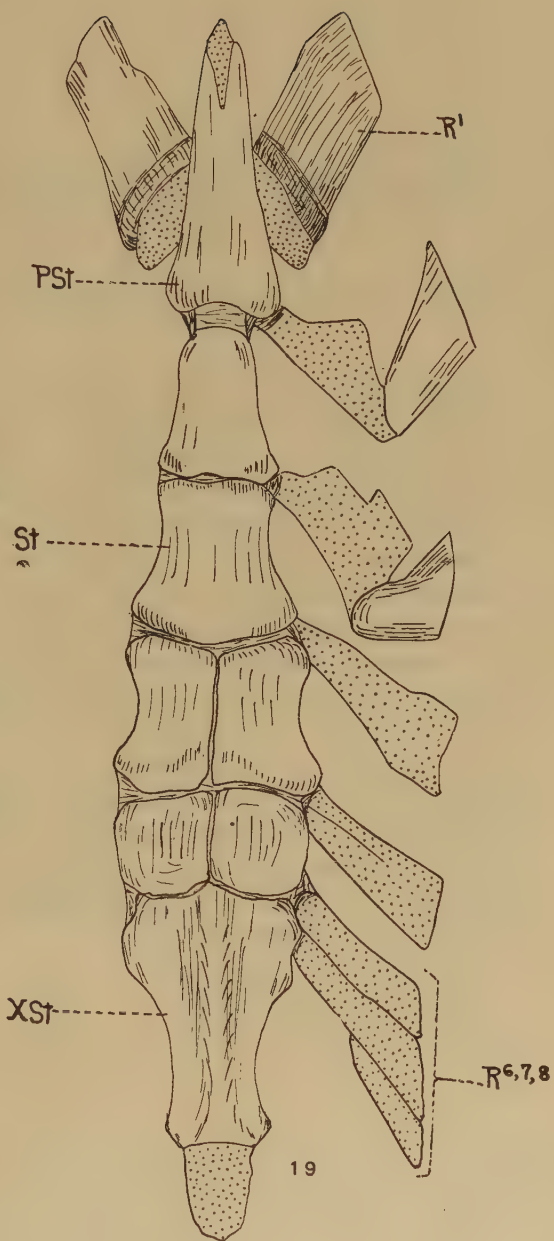
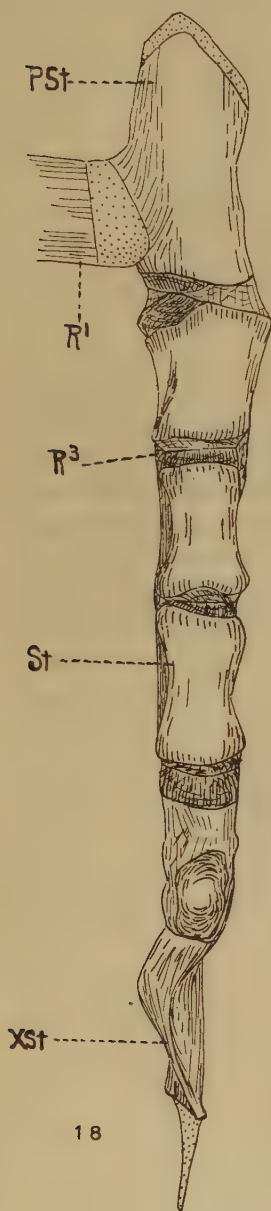


PLATE 6

EXPLANATION OF FIGURES

18 and 19 Lateral and ventral views of sternum from same specimen as in figure 17.



Resumen por el autor, Bruno Oetteking.

Museo Americano de Historia Natural, Nueva York.

El proceso fronto-esfenoidal del zigoma y su participación en la configuración de la órbita.

El autor describe un método especial para el estudio comparativo del proceso fronto-esfenoidal en proyección lateral, así como en corte transverso medio-orbitario, orientándose los objetos en un plano que pasa por el ojo y el oído. Ha examinado los cráneos de tres razas muy diferentes (suavo, esquimal y negro) así como los de los monos antropoides. Ha observado dos formas diferentes de proceso; en una de ellas éste es bastante derecho y simétrico bilateralmente (gorila, orangután), mientras que en la otra es curvo posteriormente (Hylobates, chimpancé, hombre), además de presentar diferentes grados de recesión. Esta última forma está más pronunciada en los antropoides, especialmente en el orangután y gorila, dando lugar a la "frontalidad de la órbita" (Autor). El corte transverso medio-orbitario demuestra una erección pronunciada del proceso en sentido horizontal, la cual está combinada con su mayor recesión, en vista lateral, en el hombre. Tal correlación no ha podido establecerse de un modo indudable en los antropoides.

Translation by José F. Nonidez
Carnegie Institution of Washington

THE PROCESSUS FRONTOSPHEOIDALIS OF THE ZYGOMA AND ITS BEARING ON THE CONFIGURATION OF THE ORBIT

BRUNO OETTEKING

Museum of the American Indian—Heye Foundation

FIVE FIGURES (THREE PLATES)

The orifices of the visceral or facial part of the skull, the apertura piriformis or the osseous nose, and the orbita, both are products of different cranial bones uniting and leaving between them those typically formed orifices. Taking under consideration the entrance to the orbit especially, we find that the inferior and lateral borders are formed to the larger extent by the os zygomaticum or zygoma, the malar bone after the older nomenclature. It is the processus frontosphenoidalis of the zygoma, forming the lateral border and part of the septum that separates the orbital cavity from the temporal fossa, that I wish to discuss here.

I have examined skulls of three distinctly different races: a Swabian of modern Europe, an Eskimo, and a negro, and for comparison's sake those of our next-related anthropoid forms: Hylobates, orang-utan, chimpanzee, and gorilla. The objects were orientated on the ear-eye plane and examined in exact sideward or lateral projection and in horizontal midorbital cross-section.

As is the rule in craniometry, the left orbit only was considered in the following studies.

LATERAL PROJECTION OF THE ORBITAL REGION

Instruments for orthogonal projection not being available, I used the camera lucida for outlining the parts under consideration (fig. 1). In order to avoid as much as possible distortion of

proportions by using this device, I first drew the sagittal perigram of each skull, and projected into it the measuring points of the orbital height and width (fig. 2). But instead of using the measuring points for the width as advised in craniometry (maxillofrontale and ektokonchion), I selected those points at the medial and lateral border of the orbit that are secured in bisecting the orbit parallel to the ear-eye plane. The medial measuring point will then generally fall a little below the maxillofrontale and the lateral one a little above the ektokonchion. In the proposed line of work better results are obtained by exact geometrical proportions. The fixation of the ear-point must not be omitted here for the sake of orientation.

In order to analyze the peculiar form of the processus frontosphenoidalis, a specific method of investigation had to be employed (fig. 3). It seemed practical to divide the frontosphenoidal process transversely into two halves and to ascertain the declination of their bisectors on the level that served for general orientation, viz., the ear-eye plane. A line connecting the two points of intersection of the zygomaticofrontal suture with the fore and aft border of the process serves to indicate its upper limit. The bisection of a perpendicular from the middle point of the upper limiting line just spoken of on the ear-eye plane ($E-E$) is the point through which a horizon halving the process might be laid. Connecting the bisecting points of the upper boundary (b) and the middle horizon (a) produces what might be called the 'longitudinal bisector of the upper half' of the process. A like division of the lower half is not so easily obtainable. I therefore divided the lower half again transversely laying the 'longitudinal bisector of the lower half' of the process through the bisecting points of this new horizon and the one dividing the whole process into two equal halves ($a-c$). Furthermore, a perpendicular from the forward point of intersection of the zygomaticofrontal suture will indicate the degree of concavity displayed by the anterior border of the process. I here measure the exact distance between the point of intersection of the perpendicular (p) on a line transversely bisecting the orbit ($m-l$), and the anterior border of the process (l). Taking

under consideration the entire projected distance between the medial and lateral measuring points of the orbit ($m-l$), the exact distance will show the amount of recession of the process in the different forms. An index, on the other hand, based on the values for the two distances of the transversal orbital bisecting line ($m-p$, $p-l$), expresses to what extent the concavity of the anterior border participates in the recession of the process. The higher the index, the more pronounced the concavity.

After thus resolving the frontosphenoidal process into its essential parts, a number of measurable proportions can be ascertained. From the accompanying table it will be seen that the angle formed by the longitudinal bisector of the upper half of the process and its middle horizon ($\angle b-a-h$) is, at 94° largest in the chimpanzee, a little smaller, at 85° in the Hylobates. The Eskimo, Swabian, and negro follow with still smaller values of from 74° to 61° . The behavior of the longitudinal bisector of the lower half of the process ($a-c$) is diametrically opposite. The angle considered here ($\angle a-c-E$) ranges from 75° to 93° in man, yielding 70° in the chimpanzee, and 77° in Hylobates. The two bisectors produce between themselves an angle ($\angle b-a-c$) that may be considered as angular expression of the curvature of the entire process. It is largest in the apes and shows gradual difference in man, but, strangely enough, is found with the highest value or with the most obtuse angle in the negro, who is recorded with the smallest degree of declination of the longitudinal bisector of the upper half of the process. This phenomenon is explained by the gradual but pronounced erection of the longitudinal bisector of the lower half of the process. The angular position of the frontosphenoidal process in toto on the plane of orientation will best be recognized by an angle formed by a line combining the points b and c of the same figure 3 and the ear-eye plane ($\angle b-c-E$). Here we find the apes fairly uniform, with the exception of Hylobates with an angle of 94° , while the others maintain 103° and 107° . A gradual difference will be noticed in man, with a range of from 73° over 82° to 89° .

Comparing, now, the lateral border of the orbit, as represented by the anterior border of the frontosphenoidal process to the medial one, decided differences will be noticeable (figs. 1 and 2). The exact length of the horizontal line bisecting the orbit and representing the laterally projected distance between its medial and lateral borders ($m-l$) is most significant in man. It amounts to 9 mm. in the Eskimo, 13 mm. in the Swabian, and 16 mm. in the negro. It is almost alike in Hylobates and gorilla with 7 mm. each and 9 mm. in the chimpanzee. An exceptional condition is illustrated in the orang-utan where the medial measuring point of the orbital width disappears behind the lateral one in lateral projection, to the extent of 2 mm., expressed in our list as '-2 mm.' The concavity of the anterior border as indicated by the points $p-l$ is measurable only in the Hylobates, where it amounts to 0.5 mm. In the other apes the perpendicular falls short of the orbital aperture. But there is a gradual ascent in man with 2 mm. in the Eskimo, 3 mm. in the Swabian, and 4 mm. in the negro. The indices based on these two values amount to 7.1 in the Hylobates, 22.2 in the Eskimo, 23.1 in the Swabian, and 25.0 in the negro.

The conclusions that can be derived from the measurements just discussed might be summarized as follows:

There are two distinctly different forms of the processus frontosphenoidalis in the objects investigated (fig. 1), the one of a straighter and bilaterally symmetrical shape (orang-utan, gorilla), the other curved with a forward concavity and a backward convexity (Hylobates, chimpanzee, man).

The conditions in the Hylobates and chimpanzee are not so pronounced as those in man, but clearly foreshadow them.

The angular position of the upper half of the process on a coordinate of the general plane of orientation ($E-E$) seems to be influenced by the recession of the process to such a degree as to render the angle of declination in man proportionately and gradually smaller. Hylobates (85°) remains relatively nearest to human conditions, while chimpanzee (94°) exceeds them considerably. The responsibility for this condition in the apes must without doubt be laid to the degree of prognathism, which is

decidedly less in *Hylobates*, thus securing a more upright position of the process in the latter. This is also expressed in the angular position of the lower half of the process with a more erect one in *Hylobates* (77°) and a more slanting one in chimpanzee (70°). In man there is clearly noticeable a tendency to greater uprightness. But here prognathism is eliminated as an influencing factor. The cause must rather be sought in the gradual recession of the process and the changes involved therein. The two predominating factors, then, underlying the changes of angular position of the process are *prognathism in the apes and gradual recession of the process as a racial trait in man*. This is also demonstrated by measurements 4 and 5 (and consequently 6 and 7). It is thus shown that as far as the recession is concerned the European maintains an intermediate position, while it is least pronounced in the Eskimo and most in the negro. An exceptional position in this respect is held as stated above (p. 28) by the orang-utan where the 'frontality' of the orbit—as I have termed it in earlier publications—is carried to an extreme, since the lateral border of the orbit exceeds the medial one by 2 mm. ('-2 mm.' in the tables) in lateral projection.

It may be vain to speculate on the causes of such differences in form. Nevertheless, there are certain facts that tend to justify the assumption of functional adaptations. The Eskimo living in his wide snow-bound regions of the globe has always been an 'eye-man,' while it seems that the negro is and has been a 'nose-man,' both being forced to give decided preeminence to the use of these special sensory organs in the struggle for life. The muscular attachments of these organs and the mimetic musculature of the face affected by them may have cooperated in producing these specified racial types.

THE HORIZONTAL MIDORBITAL CROSS-SECTION

Observations are made here on the perigrams of the midorbital line. The frontal plane (figs. 4 and 5, *F-M*) covers the two lateral measuring points of the orbits, and stands perpendicularly on the sagittal plane (*M-S*). Here again the left side only was considered. Most important in this connection seemed to

be the angular position of the process in cross-sectional representation, i.e., the angle formed by a line connecting the intersecting points of the fore and aft border of the process and the frontal plane as given by the points $p-l-M$. Of further importance is the angle of declination of the orbital width on the frontal plane. It is expressed by the letters $m-l-F$. Interesting diversities of form are again revealed by both of them. While there is apparently only a small difference in the first angle ($\angle p-l-M$) between the Eskimo at 50° and the Swabian at 52° , one should keep in mind that small proportions were being measured and in such discrepancies are much more telling than in larger ones. Decidedly different is the angle in the negro at 58° . In this respect also within the human range (50° to 58°) falls Hylobates at 51° , while a gradual ascent is to be found in the other apes: orang-utan at 17° , chimpanzee at 33° , and gorilla at 36° . The angle of frontal declination of the orbit ($\angle m-l-F$) amounts to 12° in the Eskimo, 15° in the Swabian, and 24° in the negro, expressing in another way the recession of the fronto-sphenoidal process. The exceptional position of the orang-utan in this respect is also manifested by the angle, yielding -3.5° . Hylobates at 17° as well as chimpanzee at 33° fall entirely within the human range, while gorilla only rises to 9° .

Here significant conclusions naturally present themselves. The measurements show that in *man the more pronounced recession of the process combines itself with its proportionately pronounced erection in the horizontal sense*. This status cannot be confirmed throughout in the apes, with the exception of the Hylobates, who shows entirely human conditions. But the apparent gradation in the other apes is somewhat deceptive, as it does not correspond to similar conditions of recession. The exceptional condition here in the orang-utan with a value of -3.5° might have involved a considerably smaller angularity of the process than 17° . Again, the smaller angle of frontal declination in gorilla at 9° might have been compensated by a lesser angularity of the process than 36° , compared to the values in the chimpanzee at 14° and 33° . It may be concluded, then, that *in the apes a correlation between the frontal declination of the orbit and the horizontal angularity of the process is not demonstrated beyond doubt*.

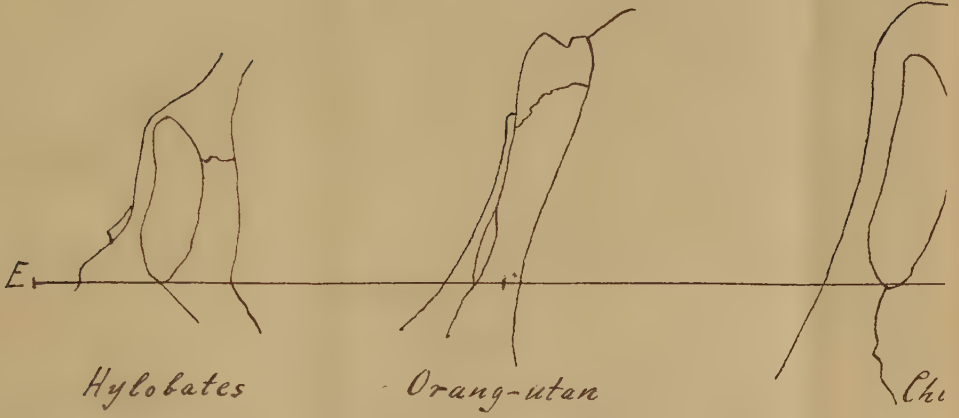
These investigations and results are based on a small number of objects only and were meant to call attention to a special field of anthropological research that can be methodically exploited: the analyzation of a morphological detail in the complexity of cranial regions.

PLATE 1

EXPLANATION OF FIGURE

- 1 Lateral projections in the orbital region. *E-E*, ear-eye plane. Natural size.

PROCESSUS FRONTOSPHENOIDALIS OF ZYGOMA
BRUNO OETTING



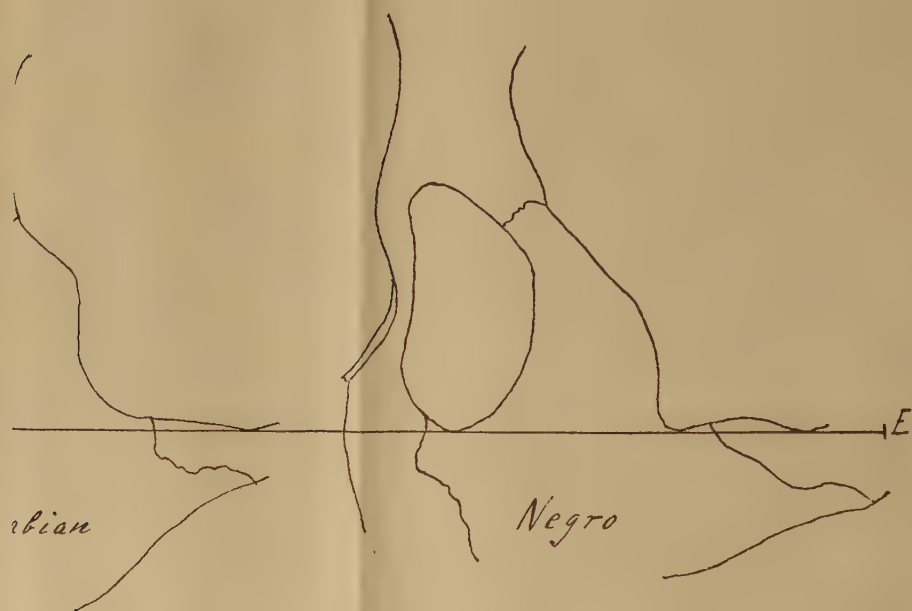
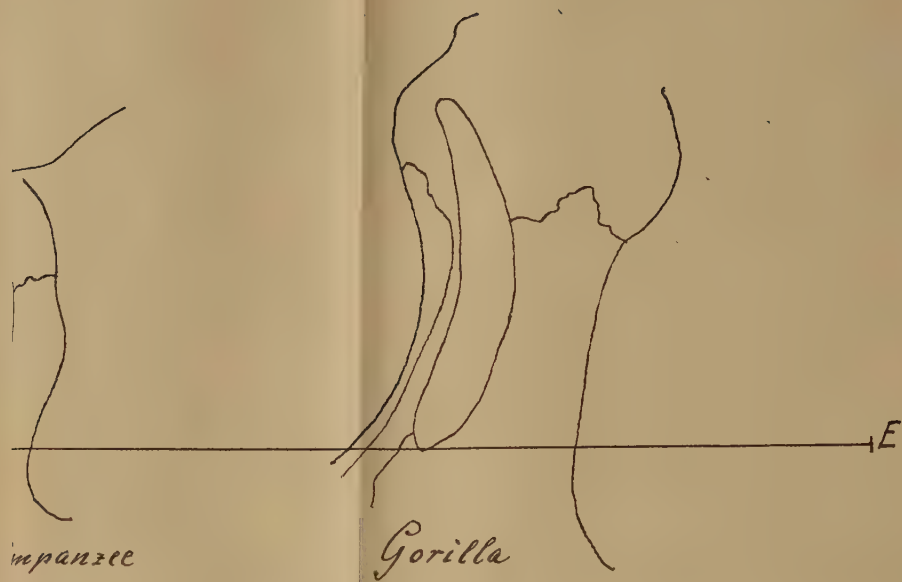


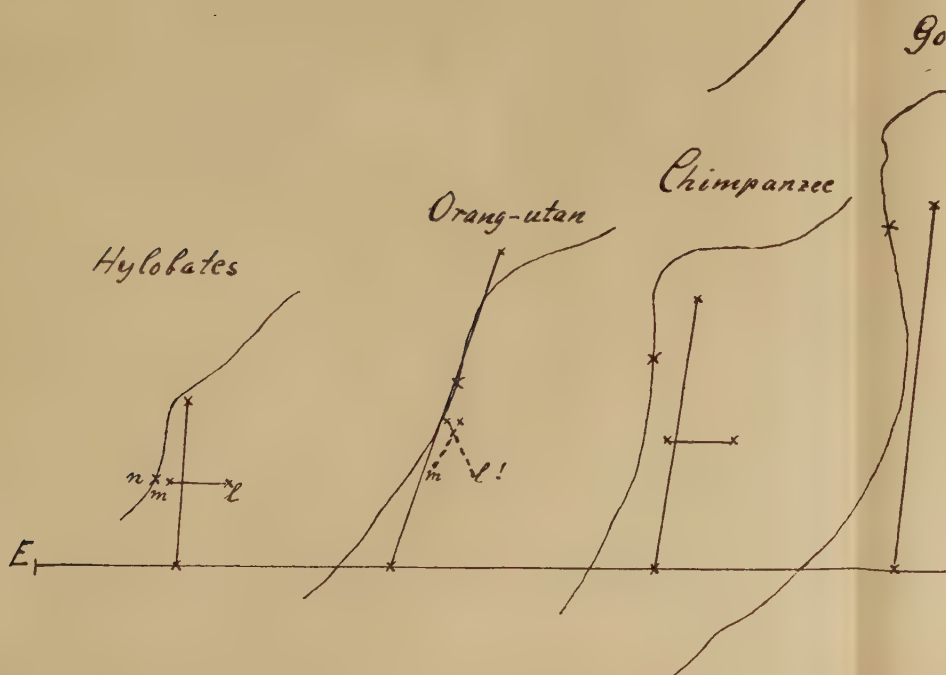
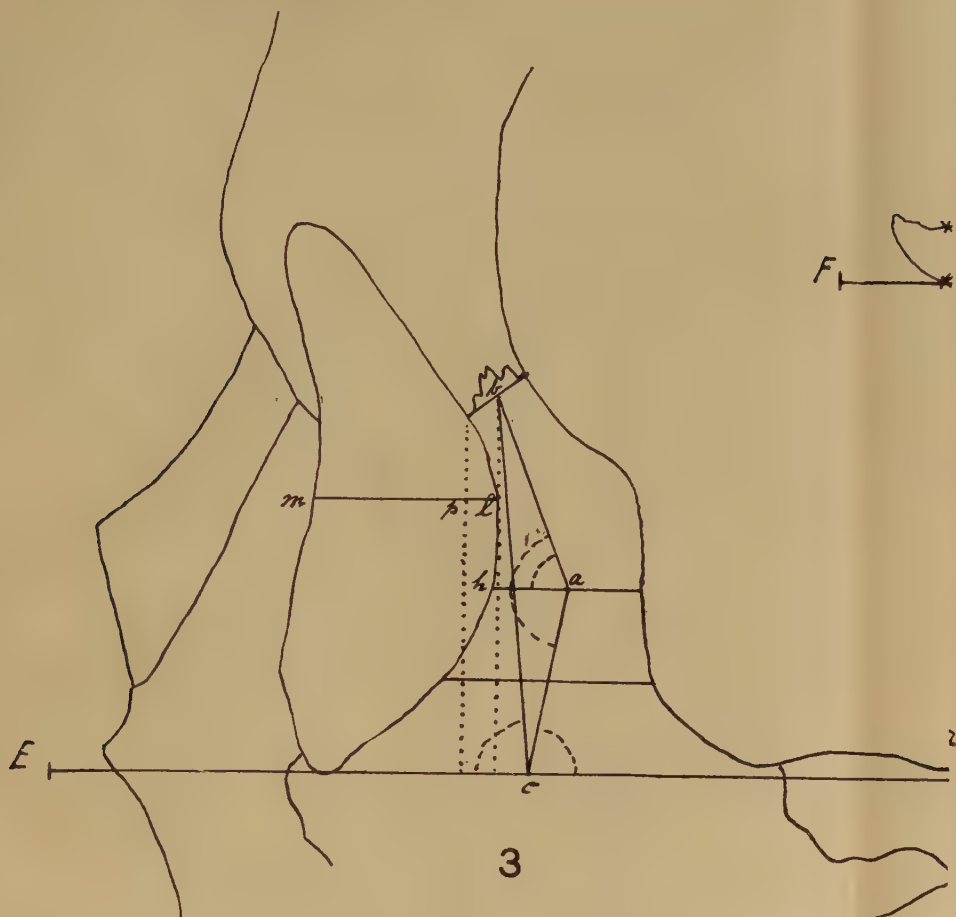
PLATE 2

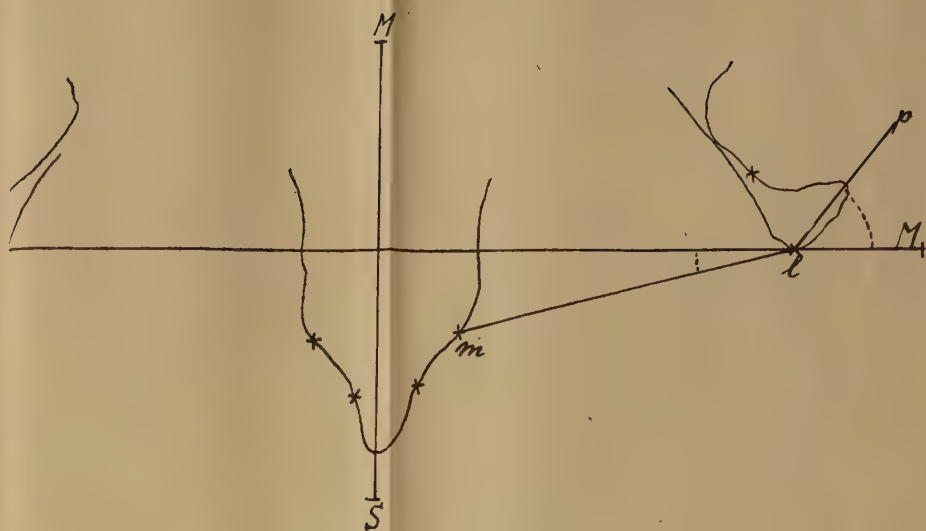
EXPLANATION OF FIGURES

2 Median-sagittal perigrams of the orbital region, the measuring points of the orbital width and height projected into them. *E-E*, ear-eye plane; *m* and *l*, medial and lateral measuring points of the orbital width; *n*, nasion. Natural size.

3 Lateral projection in the orbital region to show method of investigation. *E-E*, ear-eye plane. For other markings see text.

4 Perigram of the orbital region at the midorbital level to show method of investigation. *l-p*, line connecting fore and aft border of processus fronto-sphenoidalis. For other markings see text.





4

$\rightarrow E$

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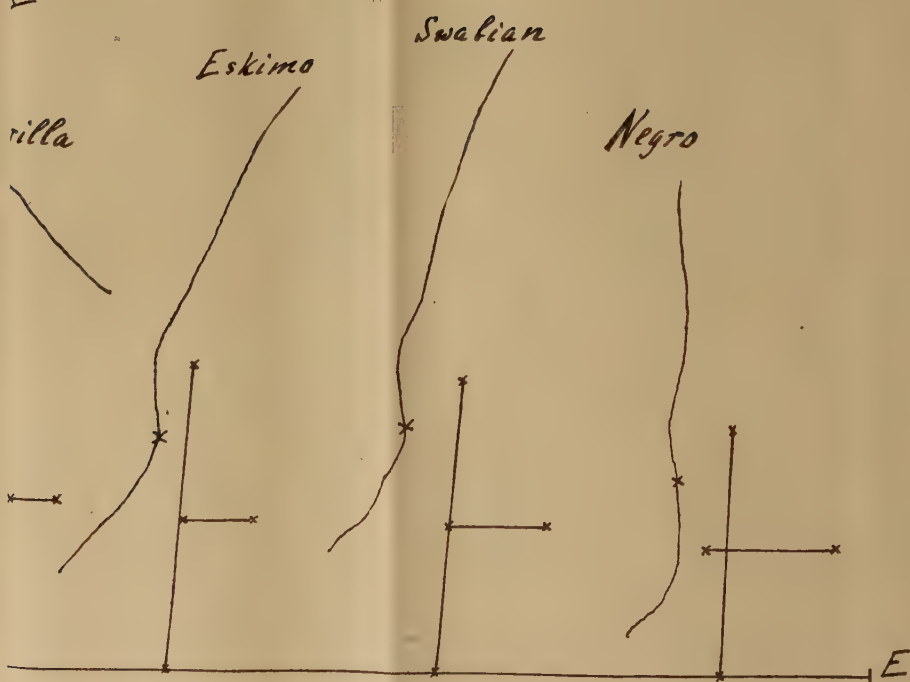


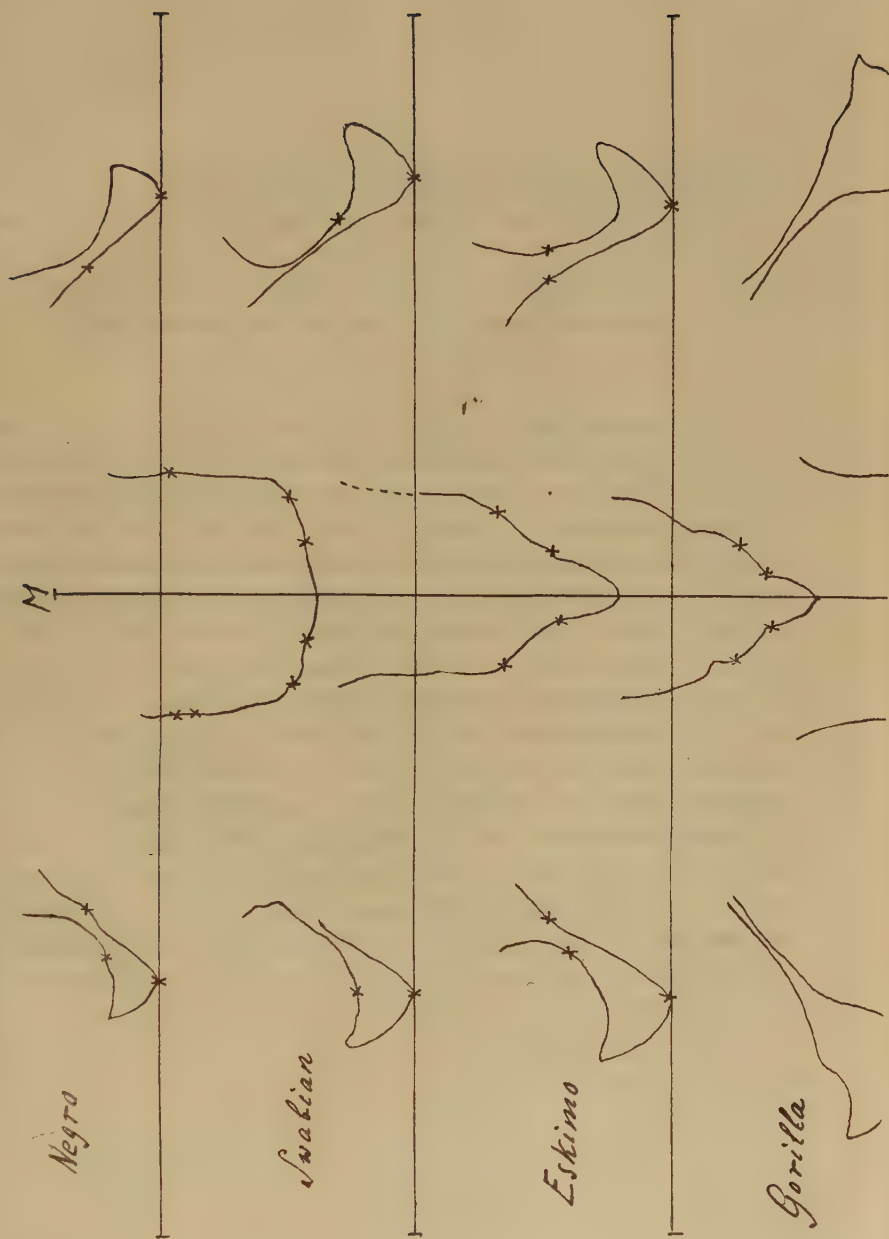
PLATE 3

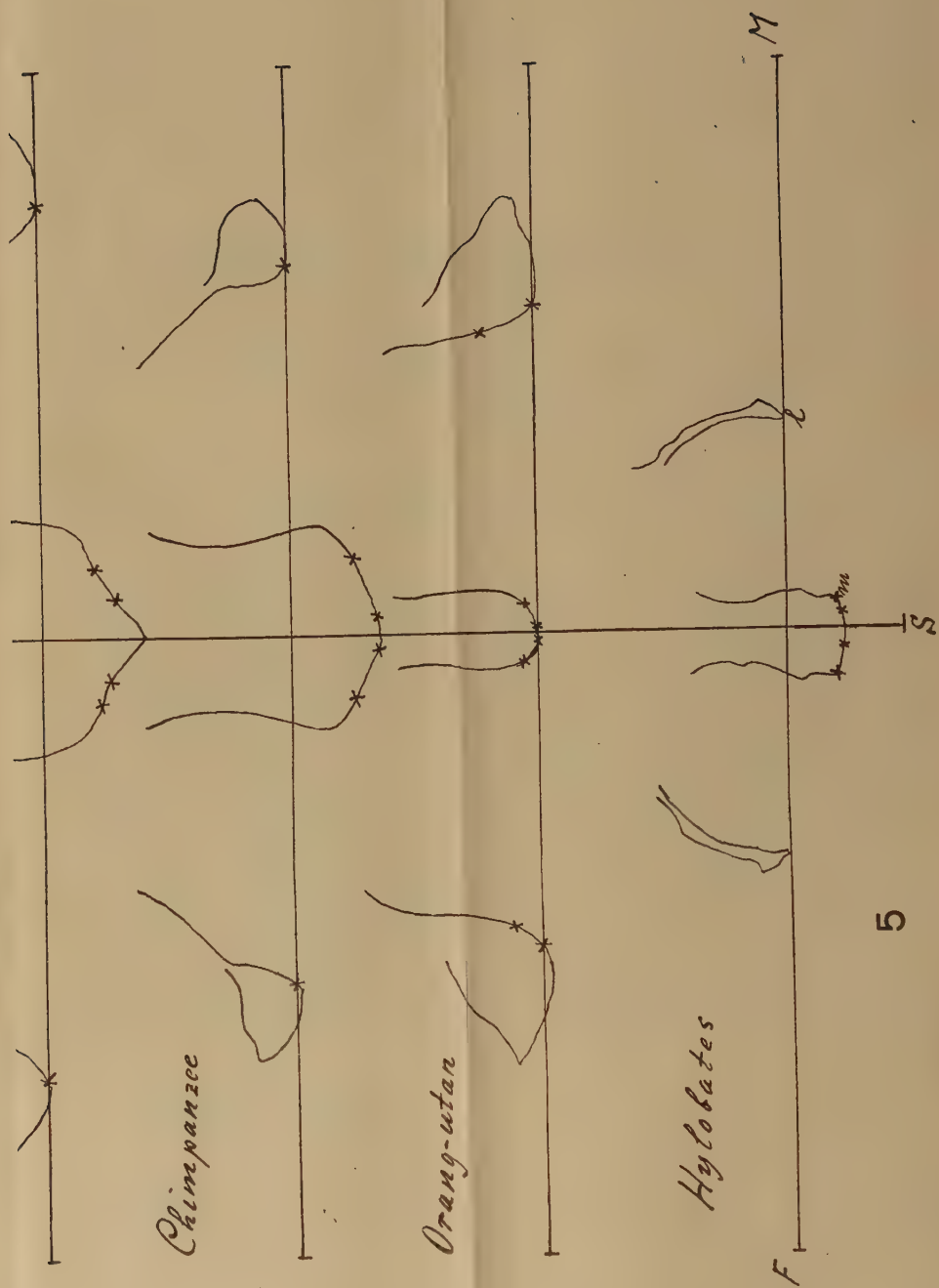
EXPLANATION OF FIGURE

5 Perigrams of the orbital region at the midorbital level. *S-M*, median-sagittal plane line; *F-M*, midorbital horizon; *m* and *l*, medial and lateral measuring points of the orbit. Natural size.

Table of measurements

SPECIMEN	$\angle$ b-a-h	$\angle$ a-c-E	$\angle$ b-a-c	$\angle$ b-c-E	ORBITAL WIDTH (m-l) PROJECTED	CONCAVITY OF LATERAL BOR- DER (p-l) PRO- JECTED	$\frac{p-l}{a-h} \times 100$	$\angle$ m-l-F	$\angle$ p-l-M
Hylobates.....	85	77	162	94	7	0.5	7.1	17.0	51
Orang-utan.....	—	—	—	107	-2	—	—	-3.5	17
Chimpanzee.....	94	70	164	103	9	—	—	14.0	33
Gorilla.....	—	—	—	103	7	—	—	9.0	36
Eskimo.....	74	75	149	89	9	2.0	22.2	12.0	50
Swabian.....	67	83	150	82	13	3.0	23.1	15.0	52
Negro.....	61	93	154	73	16	4.0	25.0	24.0	58





Resumen por el autor, Matsuziro Takenouchi.
Instituto Wistar de Anatomía y Biología.

Sobre la resistencia de los glóbulos rojos de la rata albina de diferentes edades a la acción de las soluciones hipotónicas de cloruro sódico.

La resistencia de los eritrocitos de la rata albina de edad variable a la acción de las soluciones salinas hipotónicas está relacionada con el contenido acuoso del suero que rodea normalmente a dichos corpúsculos. Aunque la variación individual es considerable, cuando se considera en grupos de individuos de mayor edad los datos obtenidos demuestran que la concentración de la solución salina productora de hemólisis aumenta con la edad (el aumento más rápido tiene lugar durante los primeros cien días). La concentración de la solución salina hipotónica capaz de producir aproximadamente un 20 por ciento de hemólisis es 0.441 por ciento para los individuos jóvenes, y 0.470 por ciento para el grupo de individuos de mayor edad. Los eritrocitos de la hembra son menos resistentes, es decir responden a la acción de una solución salina hipotónica más concentrada que la que afecta al macho. La influencia de la anestesia de las ratas por el éter, antes de obtener la sangre, y el poder protector de la proteína de esta última contra la acción de la hemólisis hipotónica son factores sin importancia.

Translation by José F. Nonidez
Carnegie Institution of Washington

ON THE RESISTANCE OF THE RED BLOOD CORPUSCLES OF ALBINO RATS AT DIFFERENT AGES TO HYPOTONIC SOLUTIONS OF SODIUM CHLORIDE

MATSUZIRO TAKENOUCHI

The Wistar Institute of Anatomy and Biology

ONE CHART

I. INTRODUCTION

Though there have been published several investigations dealing with the changes in the blood according to age, as, for instance, those cited by Gundobin ('12) for man, it seemed to me that further systematic study of possible changes in the red corpuscles during the span of life was desirable.

The chemical and physical changes of the blood serum of some animals at different ages have been studied by a number of investigators. Recently Hatai ('18) published the results of his observations on the refractive index of the blood serum of albino rats during the span of life.

He confirmed several of the results of Reiss ('09) and pointed out critical periods at weaning and puberty not previously noted. Hatai ('18) finds that the refractive index of the serum of albino rats changes according to age, and in general increases with increasing age. Before suckling, however, the refractive index of the serum is higher than just after suckling. This statement confirms the findings of Reiss ('09) in his study of the human newborn. There is, however, a period of fluctuation at weaning—twenty days—and another at ninety days. After ninety days in age the refractive index of the serum slowly rises throughout the remainder of the life span, so far as examined, up to 600 days.

The water content of the serum varies also with age. It is highest at birth and becomes lower with increasing age, falling from about 96 per cent to 91 per cent.

Among other recent publications dealing with the chemical changes in the blood according to age, we may refer here to the work of Kingsbury and Sedgwick ('17) on the uric acid content of the blood of the new-born child, in which the authors state that the uric acid content of the blood is higher during the first few days of life, both relatively and absolutely, corresponding with the higher excretion of uric acid during that period. Bock ('17) reports that the placental blood tends to be distinctly higher in its amino-nitrogen content than the average mother's blood. Abderhalden and Weil ('12) state that the nitrogen content of the serum of the fetus is less than that of the mother. All these facts indicate that the blood at or before birth differs from that of later life.

In addition to the morphological changes which have been reported in the elements of the blood before or shortly after birth and in later life, it has seemed to me to be worth while to investigate in some animal the resistance of the red corpuscles at different ages, because all the facts above mentioned suggest that there may be corresponding changes in the character of the red cells correlated with the physical and chemical changes in the plasma or serum.

Following the advice of Doctors Donaldson and Hatai, I took this problem for systematic investigation, using as material albino rats of different ages.

In the literature, so far as we are aware, there are no systematic studies on the resistance, according to age, of red corpuscles to the hypotonic salt solutions, and this was the reaction which we have chosen for study.

II. TECHNIQUE AND PRELIMINARY TESTS

1. Preparation of salt solutions of varying concentration

For the determination of the degree of resistance of the red corpuscles of albino rats at different ages, the hypotonic salt solution used was prepared in the following manner:

Chemically pure sodium chloride (NaCl) was dried on a plate for two hours at 170°C. and then weighed immediately in a closed

weighing bottle. I then dissolved 10 grams of the dried salt in distilled water, making up to 1000 cc. in a volumetric flask. This gives a 1 per cent solution which may be called 'the standard salt solution.' For obtaining solutions of less concentration, the standard solution was diluted with distilled water in the proportions necessary to furnish the final concentrations which were used. These ranged from 0.55 per cent to 0.325 per cent, at intervals of 0.01 to 0.15 per cent.

2. Method of obtaining the red corpuscles from rat blood

Under slight ether narcosis, the blood was usually taken from the arteria carotis and vena jugularis by cutting both of these vessels, but in a few instances it was taken directly from the heart. The blood was collected in a wide tube containing an equal amount of 1.5 per cent sodium citrate solution. This solution had been made by dissolving 1.5 grams of sodium citrate in 100 cc. of sterile physiological salt solution, after which the mixture was sterilized.

The suspension of red corpuscles in the sodium citrate solution as thus obtained was immediately centrifuged and the supernatant fluid removed. The mass of red cells remaining was that employed in the hemolytic tests.

Using one and the same capillary pipette throughout the whole work, and cleaning it after each use with sterile water and sterile physiological salt solution, just one small drop of the mass of red corpuscles was added to each test-tube of the series, each of which contained 1 cc. of the varying concentrations of the hypotonic salt solution. All the tubes were shaken adequately after the addition of the red cells and were placed in the refrigerator.

A reading immediately after the addition of the blood-cells to the tubes is almost unnecessary, because it is very hard to determine the color of the liquid, whether laked or not, until the red cells, which are still unlaked, have settled. For a complete settling of the red cells at least two hours are needed. The final reading was made at the end of 18 hours in the ice box.

3. Notation of the resistance of red corpuscles to hypotonic salt solution

To measure the degree of the resistance of red cells to the hypotonic salt solution, Ubbels ('01) used the percentage of the sodium chloride solution. For instance, the resistance of the red cells of a pregnant beef is from 0.75 per cent (maximum resistance) to 0.43 per cent (minimum resistance). Hamburger ('97, '02) proposed the use of the reciprocal value of the concentration instead of the latter itself; the resistance of erythrocytes to a dilute salt solution being inversely proportional to the concentration of the solutions which cause the beginning of hemolysis.

In denoting the degree of the hemolysis I have used a direct statement as follows:

Complete lysis, caused by laking red cells in distilled water, is taken as 100 per cent, using a standard colorimetric scale for comparison. Dilutions of this standard solution were then made so as to have tubes showing the color values of 80, 60, 40, and 20 per cent of hemolysis. Less than 20 per cent of hemolysis was considered as a trace, except in special cases.

As we know, the tests made in this way for the several degrees of hemolysis are most accurate between a trace and 20 to 40 per cent of hemolysis. Therefore I have selected the 20 per cent hemolysis as the standard for comparison and denoted the resistance of red cells of rat's blood by the concentrations of the salt solution, which cause approximately 20 per cent hemolysis.

Before describing the main experiments, I wish to record some preliminary tests bearing on this problem.

4. Preliminary tests

a. Influence of ether narcosis upon the resistance of red cells of rat blood to hypotonic salt solutions. The fact that chloroform and ether can cause lysis of blood has been long known. It is said that there is no marked alteration in the blood corpuscles to be seen when we add narcotica to a suspension of red corpuscles in isotonic solution in the concentration required to narcotize the animal.

In somewhat higher concentrations of the narcotica, however, the blood-cells will be laked completely. According to Fühner and Neubauer ('07) and Overton ('01), the hemolytic limit for some narcotica is about ten times as much as the narcotic limit.

I made several tests to compare the resistance to the hypotonic salt solution of the red cells of rats etherized for different lengths of time, twelve minutes, thirty minutes, etc., as contrasted with the red cells from non-etherized rats of the same litters. I noticed that the difference in the average resistance of each group of rats, etherized or non-etherized, is rather a matter of individual variation, than an effect of the duration of etherization. Repeating this test several times, I became con-

TABLE 1

Showing the effect of blood protein in protecting the red cells against hypotonic salt solutions

	NON-WASHED RED CELLS		WASHED RED CELLS	
	Maximum	Minimum ¹	Maximum	Minimum
Rabbit.....	1/0.40	1/0.55	1/0.41	1/0.56
White rat.....	1/0.35	1/0.48	1/0.35	1/0.48
Sheep.....	1/0.51	1/0.69	1/0.51	1/0.70

¹ Hamburger's notation of the resistance of erythrocytes is used.

vinced that the influence of the etherization upon the resistance of red cells is negligible.

b. Tests regarding the protection of the red blood-cells by the serum protein against the action of hypotonic salt solution. According to Hamburger ('02), it is necessary to add 200 per cent of water to frog's blood, 50 to 80 per cent to the blood of beef or man, and 130 to 200 per cent to bird's blood, to cause a complete hemolysis. The protecting agencies seem to be not only the salt dissolved in the blood, but also the protein in the plasma of the blood. Testing for the protecting influence of the blood protein, Rywosch ('07) found the values in table 1, which show that the blood protein protects only slightly the red cells against the hypotonic salt solutions.

I repeated this experiment, using washed and non-washed red rat cells from the same rat, and found that the difference in the

resistance caused by washing is much smaller than the individual variation.

From the foregoing we see that the reaction of the red cells in these tests is not significantly modified by either narcosis or the removal of the serum.

III. EXPERIMENTS ON THE RESISTANCE OF RED CORPUSCLES OF THE ALBINO RAT AT DIFFERENT AGES TO HYPOTONIC SALT SOLUTION

All the albino rats which have been used in this work were obtained from the stock colony of The Wistar Institute of Anatomy and Biology, and were apparently healthy.

Owing to the limited yield of blood in the case of rats under seven days of age, which are of course small, the desired amount of blood corpuscles for one series of tests required the use of an entire litter (eight or nine individuals) and of three to six rats when they were between one to three weeks of age. When the rats became larger, a single animal sufficed.

The results of the main experiment are given in table 2 and chart 1. In the table and chart 'new-born' designates rats before they have suckled, while '1 day' means new-born rats after suckling and within twenty-four hours after birth. Special attention has been paid to obtaining new-born rats before suckling, because, as I have already mentioned, there is, according to Reiss ('09) and Hatai ('18), a distinct difference in the chemical and physical properties of the serum before and after suckling.

In the fourth column of table 2, under the heading 'Sexes combined,' the numbers in parenthesis denote the number of rats of both sexes, the blood of which was mixed to obtain the requisite amount of red corpuscles for one test. Under the heading 'Sexes combined,' in the last column, appear the simple average values of the concentration of hypotonic salt solution as obtained from rats of both sexes at a given age.

In chart 1 the black dots joined by the continuous line before 150 days and by the broken line after 150 days show the relations of these values. These data show the resistance of erythrocytes of albino rats at different ages to be rather variable; never-

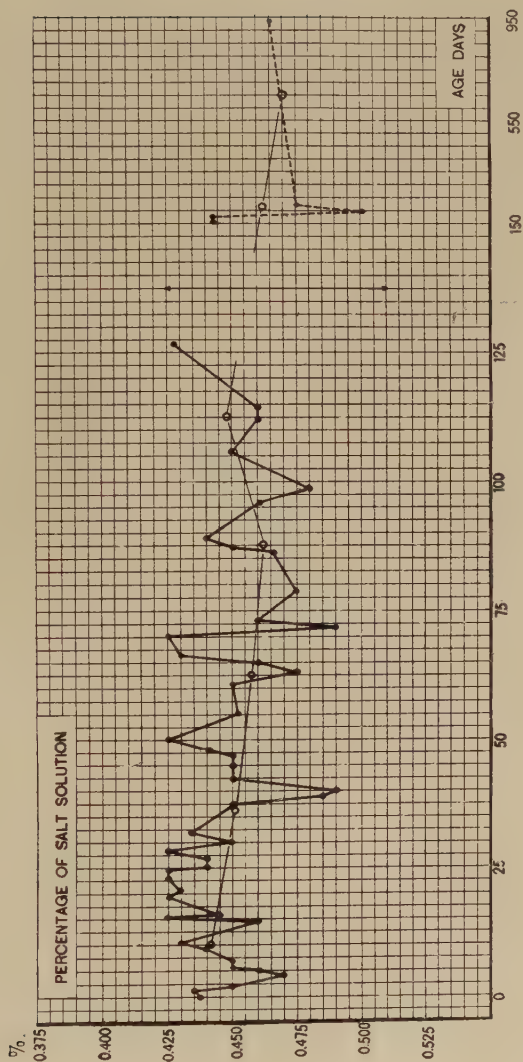


Chart 1 Showing the percentage values of the solutions of sodium chloride just producing 20 per cent hemolysis in the erythrocytes of the albino rat. The black dots joined by the continuous line, 0-125 days, and by the broken line, 150-950 days, show graphically the relations of the values given in the last column of table 2. The circles joined by the continuous line show the values as given for the age groups in table 3. After 150 days the scale for the age is only one-tenth that used for the age before 125 days, and is therefore relatively much condensed.

TABLE 2

Giving the concentration of the hypotonic salt solution which just produces 20 per cent hemolysis of the rat erythrocytes

AGE	NUMBER OF TEST ANIMALS			AVERAGE PERCENTAGE		
	Male	Female	Sexes combined	Male	Female	Sexes combined
<i>days</i>						
New-born			(8) (9) (9) (9)			0.437
1			(8) (9)			0.435
2			(4) (5)			0.450
4			(3)			0.470
5			(3) (4)			0.460
6			(6)			0.450
7			(6)			0.450
9			(6) (6)			0.440
10			(3) (3)			0.430
14			(2) (2)			0.460
15		1	(3) (3) (2)		0.425	0.425
			(2) (2) (2)			
16			(3) (3)			0.450
19			(2) (2) (4)	0.450		0.425
20	1	1	(2) (2) (3) (3)	0.425	0.440	0.433
23			(4)			0.425
24			(2) (2) (6)			0.425
25	1	4		0.425	0.445	0.435
27	1			0.440		0.440
28		1			0.425	0.425
30	2	5		0.450	0.470	0.460
32	1	1		0.425	0.440	0.433
37	1			0.450		0.450
39	1	2		0.460	0.500	0.480
40	5	2		0.495	0.465	0.480
42	1	3		0.450	0.445	0.447
45	2	5		0.450	0.450	0.450
47	1	2		0.450	0.450	0.450
48	2	3		0.435	0.450	0.442
50	2			0.425		0.425
55	3	1		0.450	0.460	0.455
61	3			0.450		0.450
63	3	1		0.475	0.480	0.477
65	2	1		0.465	0.460	0.463
66	4			0.430		0.430
70	2	1		0.425	0.425	0.425
72	1	4		0.500	0.490	0.495
73	2			0.460		0.460

TABLE 2—Continued

AGE	NUMBER OF TEST ANIMALS			AVERAGE PERCENTAGE		
	Male	Female	Sexes combined	Male	Female	Sexes combined
<i>days</i>						
78	2	2		0.475	0.475	0.475
86	2	3		0.450	0.470	0.460
87	1			0.450		0.450
88	7	1		0.430	0.475	0.453
96	2			0.460		0.460
98	10	2		0.476	0.490	0.483
106	3			0.450		0.450
112	1	1		0.460	0.460	0.460
114	1	2		0.450	0.470	0.460
128	4			0.428		0.428
179	3	2		0.430	0.465	0.447
180	3	1		0.437	0.460	0.443
195	1			0.500		0.500
225	1			0.475		0.475
947		2			0.465	0.465

theless, the values have a definite trend, for when the averages for the seven age groups are entered as in table 3 A, it becomes evident that with advancing age the concentration of the solution producing the reaction rises. This is also shown by the line which joins the small circles in chart 1.

As shown in table 2, the concentrations of the hypotonic solutions which cause just or nearly 20 per cent hemolysis of the erythrocytes in the rat lie for the most part between 0.425 and 0.475 per cent; a concentration over 0.475 per cent seems to be exceptional.

The number of male rats examined was greater than the number of females, especially in the later ages, but when the males and females in each of the twenty-three pairs of records for the same age are compared, fourteen pairs show the concentration higher for the female, three pairs show equality, and six a concentration which is lower for the female. When these data are averaged according to age groups, it appears, as shown in table

3, B, that in five out of the six age groups the concentration sufficing for the females is higher, while in the remaining group they are alike for the two sexes. From this it is evident that there is a sex difference in the response of the red cells to hypotonic salt solutions.

Although we have no precise observations on the percentage of water in the rat serum or plasma according to sex, yet there is some reason to think that in man at least the plasma has a lower water content in the female than in the male—a condition which would be in harmony with these results.

TABLE 3

Giving the concentration of the hypotonic salt solution which just produces 20 per cent hemolysis of the rat erythrocytes, based on the seven age groups which appear in table 2

A. Percentages according to age—Sexes combined.

B. Percentages according to age—Males and females separately, in twenty-three pairs of determinations.

AGE	A. AVERAGE PERCENTAGES	B. AVERAGE PERCENTAGES		
	Sexes combined	Number of pairs	Males	Females
<i>days</i>				
1- 25	0.441	2	0.425	0.443
25- 50	0.449	8	0.452	0.459
50- 75	0.457	5	0.463	0.463
75-100	0.463	4	0.458	0.478
100-125	0.449	2	0.455	0.465
175-200	0.463	2	0.434	0.463
225-950	0.470			

IV. GENERAL DISCUSSION

The resistance of the red-blood cells of albino rats at different ages to hypotonic solutions of sodium chloride show fairly regular changes. These changes correspond to those observed by Hatai ('18) in the refractive index and in the water content of the serum of the same species. Our attention is directed especially to the water content of the serum which is highest at birth and becomes lower with increasing age, falling from about 96 per cent at birth to 92 per cent during the first hundred days of life and then to about 91 per cent later.

The resistance of the red blood-cells follows a corresponding course and the young cells adapted to a serum with a high water content require therefore a lower concentration of hypotonic solution to produce hemolysis than do the old cells which were adapted to a serum having 5 per cent less water in it (Hatai, '18).

The fact that a higher concentration suffices to produce hemolysis in the females as compared with the males suggests that in the female the serum should contain less water than in the male, but at the moment there are no direct observations on this point.

In this connection it may be noted that in the literature there do not appear any systematic studies on the changes in the intraglobular water according to age, though there are some data on the water content of the erythrocytes of several mammals (Hamburger, '02; Abderhalden, '98). The results obtained by these authors are, however, very different from each other and the discrepancies still await an explanation.

Further, we do not know whether there is any regular change in the osmotic concentrations of the blood serum related to age. According to Bugarszky and Tangl ('98), the molecular concentration, or the osmotic concentration (Hamburger, '02) of the blood serum (computed from $\frac{\Delta}{1.85}$) shows only slight differences in different mammals. The individual variation in the osmotic concentration of the blood serum in any given species is from 0.10 to 0.05 Mol. per liter—differences, which, according to the authors mentioned, may be caused principally by the different nutritive conditions of the animals.

Nevertheless, it seems probable that there are some physico-chemical changes in both the serum and the erythrocytes of rat blood at different ages, even if there may not be a great change in the molecular concentration of the blood serum.

According to the unpublished results of Dr. Y. Suzuki, working at this laboratory, red cells from young rats when mixed with the serum of an old rat, show a slight agglutination, while on the other hand, red cells of an old rat swell in the serum of a young rat, sometimes enormously, and come even to complete lysis.

The adequate interpretation of our results, which show an adaptation of the red cells to the change in the water content of the serum, can be given only after suitable data have been obtained for the water content of the erythrocytes and the osmotic concentration of the serum at different ages in the albino rat.

V. SUMMARY AND CONCLUSIONS

1. The resistance of the erythrocytes of albino rats at different ages to hypotonic salt solutions is related to the water content of the serum normally surrounding the corpuscles. Although there is considerable individual variation, yet when taken in the larger age groups the data show that the concentration of the salt solution causing hemolysis increases with age—the most rapid increase occurring during the first hundred days (table 3, A). The concentration of hypotonic salt solution which causes approximately 20 per cent hemolysis is 0.441 per cent for the youngest and 0.470 per cent for the oldest age group.

2. The erythrocytes for the female are less resistant, i.e., respond to a higher concentration of the hypotonic salt solution, than do those of the male (table 3, B).

3. The influence of etherization of the rat before bleeding and the protective power of the rat-blood protein against hypotonic hemolysis are both negligible.

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Resumen por el autor, Leslie B. Arey.

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Sobre la presencia de sistemas de Havers en el hueso de membrana.

Se supone comunmente que los huesos de origen puramente intramembranoso carecen de sistemas de Havers. Puesto que la mayor parte de los huesos de cartílago se desarrollan realmente a expensas de membrana, tal afirmación es sospechosa. Cuando se trata de comprobar esta afirmación puede descubrirse que las secciones de los huesos de membrana del hombre presentan numerosos sistemas de Havers, que pueden encontrarse hasta en las trabéculas de su interior esponjoso.

Translation by José F. Nonidez
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ON THE PRESENCE OF HAVERSIAN SYSTEMS IN MEMBRANE BONE¹

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TWO FIGURES

The essential identity of the histogenetic processes involved in 'intracartilaginous' and 'intramembranous' bone development seems to be established beyond controversy. Moreover, it is evident that 'cartilage bone' and 'membrane bone' are terms useful chiefly in denoting the osteogenetic method used when a bone first begins to ossify. Except for a certain amount of cancellous bone with calcified cartilage cores, the greater part of even a typical 'long' bone is of periosteal or endosteal—and hence membranous—origin; for this reason the current terminology is patently inappropriate with reference to the definitive product. Probably the belief, in the past, in a metaplasia of cartilage into 'cartilage bone' is largely responsible for the fixation of this nomenclature, which was supposed to describe appropriately two distinct modes of osteogenesis.

Since so much of the bone of the skeleton is, in its final form, of the so-called membranous type, one might reasonably expect a uniformity of structural arrangement. Yet the idea is rather widespread that those bones, which from the first form in membrane, lack Haversian systems. Certain texts and writings clearly imply this; in other standard histological texts are found such statements as: "The secondary deposit of Haversian lamellae, however, never takes place, the conspicuous systems of concentric layers being absent in membrane bones," or "Membrane bones lack Haversian systems."

It is credible that the cancellous bone of the skeleton may lack Haversian systems; in light of its developmental history, this

¹ Contribution no. 71, April 1, 1919.

absence need cause no astonishment. But the quotations just cited, when interpreted, imply that compact bone possesses or lacks Haversian systems, depending on the presence or absence of a former neighboring and provisional mass of cartilage which enters into no significant relation to osteogenesis. On purely theoretical grounds, this curious statement demands critical attention.

When the matter is put to actual test, it is found that sections, ground from bone of purely intramembranous origin, demonstrate clearly the presence of numerous typical Haversian systems. My preparations show them in such bones of man as the parietal, frontal, zygomatic, vomer, palate, maxilla, mandible, and the squamous portions of the temporal and parietal. The general appearance of periosteal lamellae, Haversian systems, and interstitial lamellae, especially in the more massive regions, resembles the classic picture familiar in 'long' bones (fig. 1).

In elongated bones or processes, as, for example, the mandible or the zygomatic process of the temporal, the Haversian systems course chiefly parallel to the long axis. In the flat bones the general disposition is parallel to the surface, and, apparently, with a preponderating tendency toward a radial arrangement.

Moreover, the statement, sometimes encountered and more often implied, that cancellous bone lacks Haversian systems, is not unqualifiedly true. They may be present or absent in membrane bones. Especially where trabeculae are coarsely developed are Haversian systems likely to be found (fig. 2); such true Haversian systems, however, are not to be confused with the concentric lamellae frequently seen in spongy bone bounding marrow spaces (figs. 1 and 2, *x*).

The foregoing account controverts those current statements which pertain to the absence of Haversian systems in membrane bone. Such an erroneous belief has served to perpetuate a false histological distinction between bones primarily of intra-cartilaginous and intramembranous origin. On the contrary, in the arrangement of bone tissue into periosteal, Haversian, and interstitial lamellae, there is essential architectural uniformity, irrespective of the mode of development.

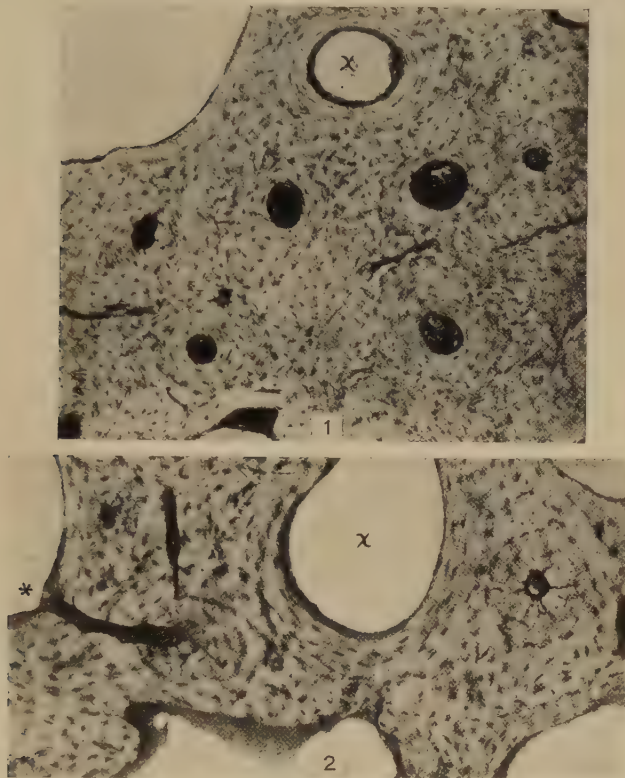


Fig. 1 A ground section from the temporal bone of man. Photograph. $\times 80$. The plane of the section passes transversely through the zygomatic process. Eight Haversian systems and several series of interstitial lamellae appear in the field. At the upper right and left corners are portions of the marrow cavity. The space marked x is apparently a pocket of the marrow cavity; although surrounded by concentric lamellae, such a complex is not properly included with true Haversian systems.

Fig. 2 A ground section of cancellous bone from the diploë of the parietal bone of man. Photograph. $\times 100$. Within the trabeculae are three typical Haversian systems; a star indicates where an Haversian canal of one passes into the marrow cavity. The marrow space, x , is enclosed by concentric lamellae; this association cannot be mistaken for an Haversian system and does much to explain the true nature of the complex shown at x in figure 1.

Resumen por el autor, John S. Latta.

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La morfología de los llamados balancines en ciertas especies de
Amblystoma.

El balancín es un apéndice largo, viliforme, que aparece a cada lado de la cabeza, en posición algo ventral al ojo y equidistante de este y de la base de las branquias externas. Alcanza el máximo de su longitud cuando la larva mide próximamente de 13 a 14 mm. Su función, que según se ha demostrado mediante una serie de experimentos, consiste en mantener la cabeza a cierta distancia del fango del fondo, se sustituye por la de los miembros anteriores en vías de desarrollo, y el balancín, no sirviendo mas para este fin, se rompe. Un estudio interno del balancín demuestra que se origina en un punto casi puesto a la articulación del cartílago de Meckel con el cuadrado. En el mesenquima de esta región se desarrolla una delgada placa de hueso dérmico, extendiéndose dentro del balancín en forma de cilindro hueco, proyectándose su extremo proximal en el tejido subyacente, en dirección del cartílago cuadrado. Este hueso no parece tener homólogo en otras especies y se considera como el resultado de la necesidad mecánica de la rigidez. Después de desprenderse el balancín persisten restos de hueso, pero se absorben finalmente. En varios intentos para establecer la homología del balancín con estructuras presentes en otras formas, se ha comparado dicho órgano con una branquia externa modificada, y también se ha considerado como el homólogo del tentáculo de los Cecílicos. A causa de las diferencias en los rasgos estructurales, desarrollo y relaciones tales homologías son imposibles. Si este órgano tiene un homólogo en otras formas animales es mas probable que represente los "discos chupadores" de Triton y los órganos viscosos de las larvas de anuros, los cuales han adquirido aquí un pedún culo.

THE MORPHOLOGY OF THE SO-CALLED BALANCERS IN CERTAIN SPECIES OF AMBLYSTOMA¹

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FOUR FIGURES

The larvae of certain species of the genus *Amblystoma* and a few other salamanders are, at an early stage of their development, possessed of a long villiform process on each side of the head, a little ventrad of the eye and equidistant between it and the base of the external gills (fig. 1). These processes are very rigid and resistant for structures so slender and are almost immovable. When fully formed they point outward and downward from the body. The present study was begun as an attempt to elucidate the structure of this organ and to discover if possible its homology and true functional rôle.

Shortly after the appearance of the gill and arm buds in the *Amblystoma* larva, the balancers become noticeable as a prominence on each side of the neck region in the location mentioned above. From these prominences are developed the long cylindrical villiform processes which, because of their rough resemblance to the balancers of dipterous insects, were so named by Clark in 1880. The balancers are prominent at the time the embryo escapes from the surrounding jelly, having acquired considerable length and assumed the characteristic cylindrical shape which is maintained until they are shed.

Several theories have been set forth to account for the function and significance of the so-called balancers. Clark ('80),

¹ Acknowledgments are due the Department of Zoology, Cornell University, where these observations were made, to Dr. H. D. Reed for help and suggestions, also to Professor S. H. Gage for the use of several series of *Amblystoma* larvae.

after observations upon the larvae, noted that as the larva approached the bottom of the pool or dish, covered with a deposit of light vegetable debris, owing to the position of the balancers, sinks but slightly into the ooze, above which the head and gills are held free and readily accessible to a supply of clearer water. Clark also suggests that a further advantage to the animal is found in the elevation of the pericardial region above the substratum, thus diminishing interference with the beating of the heart. Both of these suggestions appear plausible from observations which the present writer has made.

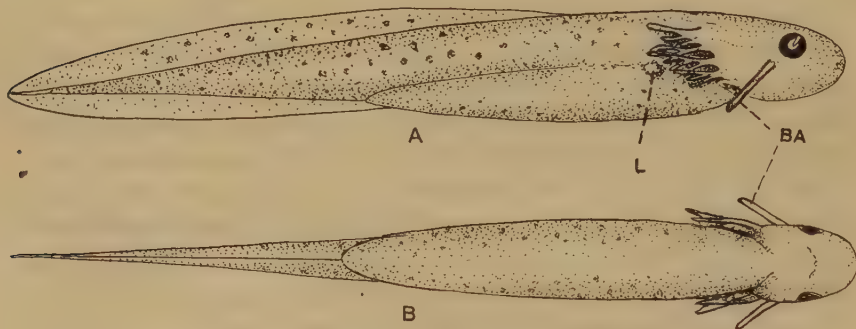


Fig. 1 *a*, Larva of *A. punctatum*, 10 to 11 mm., side view. *b*, same, ventral view. *BA*, balancer; *L*, developing fore limb.

Clark makes no statement of a belief as to the homology of these structures. Cope ('89) compares the balancers of Amblystomids with the tentacles of the Caeciliidae. He observes that the latter group has been derived from the leg-bearing Urodeles through forms represented by the Amphiumidae by a process of degeneration. The Caeciliidae are subterranean. Associated with their life in a dark abode, there is developed a peculiar, sensory tentacle on each side of the head issuing from a canal in the maxillary bone, within which it may be retracted. The tentacle appears externally between the orbit of the eye and the nostril. Cope suggests that this tentacle is homologous with the balancers of Urodeles.

To link these tentacles with the urodelan balancer, I looked for traces of a tentacular system in *Amphiuma*. Davison ('95) describes a rudimentary tentacular system in *Amphiuma* which Kingsley ('96) has pointed out as being due to a misinterpretation arising out of the presence of a trematode in the subocular blood-vessel. A careful examination of several series of sections of embryos, larvae, and adult *Amphiuma* revealed no trace whatever of a tentacular system. In the region of the balancer there are two or three folds of the integument which in transection resemble balancers in superficial features only. These folds or ridges are quite likely to have resulted from methods of fixation and hardening. They are apparently wholly without significance.

Kingsley further observes that no homology exists between the Caecilian tentacle and the Urodelan balancer, since the latter apparently represents a modified external gill, while the tentacle can be nothing of the sort. The larvae used in this study were of the species *Amblystoma punctatum*, with the exception of a single specimen of *A. opacum*. Observations upon this material substantiated Clark's statements that the lobes from which the balancers develop arise either simultaneously with the branchial lobes or a day or two later. At the time of hatching, the balancers are quite prominent, being at this period about two-thirds of their maximum length. An increase in length continues until the larvae attain a length of 13 to 14 mm.

Extending to and from the free end of the balancer there is a central artery and vein in which the blood circulates precisely as in a filament of gill. About the time the larva attains a length of 14 mm. the balancers have reached their maximum size and begin to decrease in diameter, this being noticeable in the resulting slenderness. The circulation becomes less rapid and finally almost entirely ceases. In the meantime the arms have reached a state in their development which enables them to support the body, thus rendering the balancers as useless appendages on the side of the face region. Apparently being of no further use, the balancers are lost. In case of other larval parts not persisting in the adult, absorption is the mode of disappearance, but the balancers of *Amblystoma* are broken off.

Clark has seen a larva in the act of breaking off the balancers. This was done by repeatedly shaking the head until the appendage loosened at its base and finally separated from the head. Isolated specimens in this laboratory have been observed with a balancer on one side and none on the other, while the detached balancer was found at the bottom of the container. Without such observations even, there is evidence that the balancers are cast off rather than absorbed. The reduction in the size of the balancer is found in its diameter only, the maximum length being retained up to the time of disappearance. Furthermore, larvae are frequently seen with very long balancers on one side and none on the other.

The internal anatomy of the balancer shows its relation to the other parts as a growth from the side of the throat at a point opposite and somewhat anterior to the articulation of Meckel's cartilage with the quadrate. Superficially, one is inclined to regard the balancer as a vestigial or modified external gill. But a thorough study of the region and the relation of this appendage seems to justify a different conclusion.

As is well known, there is a certain definite relation existing between the branchial cartilages and the bases of the external gills. At no time in its development does the balancer show evidence of such a relationship with either the hyoid or Meckelian cartilages, being widely separated from them from the time of its appearance by a considerable amount of mesenchyme (fig. 3). Blood-vessels within the balancers themselves are similar to those of a gill filament, but this fact alone is not sufficient evidence upon which to base an homology with a gill. The relation of the balancer vessels to those of the aortic arches is not that of external gills. If the balancer were a modified or vestigial gill, at some time in the course of its development it would be expected to bear a similar relation to the hyoid or Meckel's cartilage as that of the external gills to the gill arches. As there is no such similarity, this view loses some of its support.

By the time the larva is 9 mm. long a thin plate of dermal bone is seen to have formed in the embryonic connective tissue directly underneath the epidermis. This extends out into the balancer

as a thin cylinder. In *A. punctatum* this hollow core of bone extends nearly to the end of the balancer. As the balancer increases in length and circumference the bone becomes thicker and heavier and finally extends throughout its entire length. The proximal ends of the bone project from the base of the balancer into the underlying tissue (fig. 2).

In the species *A. opacum* the bone is found, at time of full development, to be somewhat thicker and heavier than in *A. punctatum*.



Fig. 2 Section of a larva of *A. opacum*, 16 mm., in the region at which the balancer is joined to the head. *B*, balancer; *BB*, dermal bone which develops in connection with the balancers; *M*, mouth.

tatum. It is not merely a hollow cylinder in this species, but spicules of bone project into the hollow center, thus giving it added strength (fig. 3). On the other hand, the bone does not continue throughout the entire length of the balancer, being confined to the proximal portion. The balancer in *A. opacum* is approximately the same length as in *A. punctatum*.

This bone, by virtue of its development and location, must be considered as one of the dermal system so numerous in the head region of vertebrates. It is obviously without homologue in

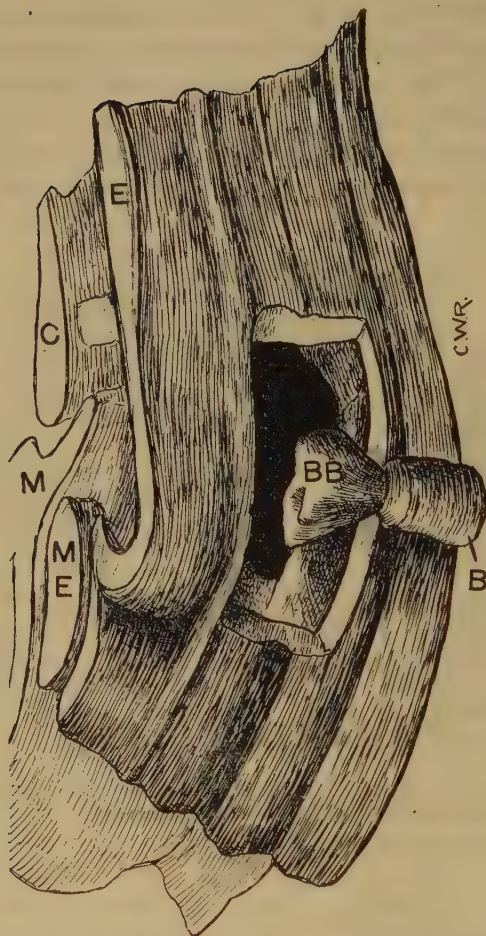


Fig. 3 Model of that region of the head of *A. opacum*, 16 mm., at which the balancer is joined to it. *B*, balancer; *BB*, dermal bone of the balancer; *ME*, Meckelian cartilage; *M*, mouth; *C*, cartilage surrounding the neural tube; *E*, epidermis.

other species and can be considered only as a response to mechanical needs in furnishing rigidity to the balancer.

Cope ('89), as stated above, has suggested an homology between the tentacle of Caecilians and the balancers of Urodela. The Caecilian tentacle in development arises from the anterior part of the orbit of the eye and migrates in development, downward and forward to a position on the upper jaw. During this downward growth the maxillary bone forms first a furrow in which the tentacle is lodged and finally a complete canal which is traversed by the tentacle. This study has revealed no evidence of a migration of the balancer of Urodela or any structural relation to the orbital region, as one would expect were the balancer a modified Caecilian tentacle. Furthermore, bone found in the balancer bears an entirely different relation to it than does the maxillary bone to the tentacle. The bone of the balancer is formed within its own substance, while that of the tentacle is independently formed and secondarily comes into relation with it. Cope's view seems untenable.

In Anuran larvae there is found a viscid organ in early stages on the ventral side below the hyoid arch. In Triton, a European urodele, viscid organs are found in the same relative position, but differ from those of Anura by possessing a stalk. Balfour has suggested the existence of similarity between these stalked suctorial processes of Triton and the balancer of Amblystoma. The loss of the 'suctorial disc' and the acquisition of the bone in the balancer coinciding with the change in function.

The behavior of the larvae was observed to ascertain, if possible, the part the balancers play in the life of the organism. The support of the head above the sediment of the bottom is clear. They have also been seen to hang freely suspended by means of these appendages upon blades of grass, twigs, and shreds of vegetable material in the water.

Larvae from which the balancers were removed suffered no ill effects in a constitutional way. Experiments and observations were made with a large number of larvae from which the balancers were removed. It was observed that when the larvae approached the bottom in this balancerless condition, they sink

into the ooze which so covers the gills that respiration is necessarily hindered. Not only do the balancerless individuals sink into the ooze when coming to rest, but the momentum gathered in swimming diagonally toward the bottom carry them into the mud so as to partly bury the head and gills. This does not occur in individuals possessing uninjured balancers. When the water was agitated even only slightly the larvae deprived of balancers were unable to maintain an upright position, being

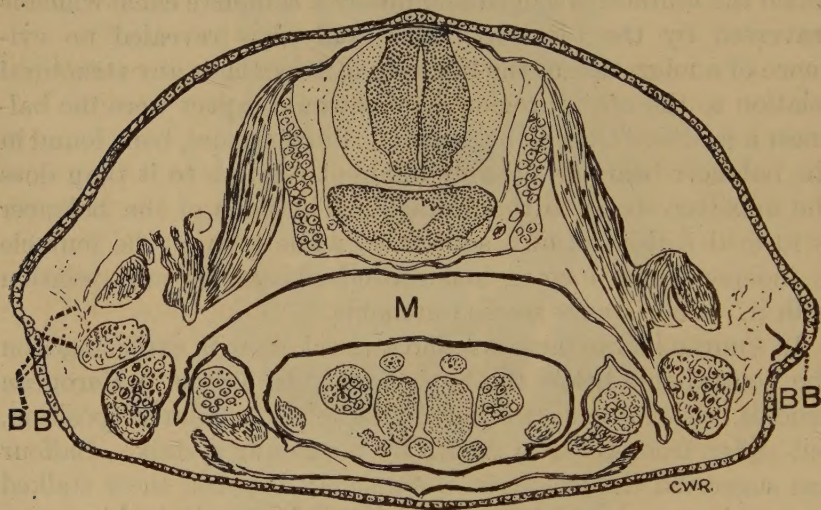


Fig. 4 Section of a larva of *A. punctatum*, 20 mm., in region after it has been dropped from the body. *M*, mouth; *BB*, remnants of the bone of the balancer, which have persisted after its disappearance.

easily turned on the side. As a result, the gills are pushed into the mud and movements to free that side resulted in entangling the gills of the opposite side. Normal individuals maintain a steady position under the same conditions where the use of the balancers as props is clearly demonstrated. Curiously, the balancers possess some regenerative powers. In a few cases after removal these structures were noted to increase in size. It is interesting to note in connection with the dermal bone of the balancer, that remnants of it are found in the underlying tissue for some time after the shedding of the balancer (fig. 4).

It appears that the bone is broken off at the base of the balancer at the time of its shedding, leaving the basal part projecting into the underlying tissue in the form of a funnel or hollow truncated cone. The size of this remnant varies somewhat, but it always persists for a short period. This is not significant, however, as the bone remnants take no part in the formation of any bone structures in this region of the adult, but remain in the same position until they disappear through absorption.

Because of its position and of its dermal origin, the bone of the balancer might be looked upon as a derivative of a branchiostegal structure. An examination of recent literature upon this subject (Allis, '18) leads one to believe that no such homology exists.

Because of differences in structural features, development, and relations, it seems impossible to homologize the balancer with either an external gill or the Caecilian tentacle. If the balancers of Urodele larvae have any homologue in other animal forms, it is most likely the stalked 'suctorial discs' of Triton and the viscid organs of Anuran larvae.

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